GENETICS

A periodical record of investigations bearing on heredity and variation

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VOLUME 45 APRIL, 1960 NUMBER 4

TABLE OF CONTENTS

SHAPARD, PAULINE B., A physiological study of the vermilion eye color mutants of *Drosophila melanogaster* 359

MILKMAN, ROGER D., The genetic basis of natural variation. II. Analysis of a polygenic system in *Drosophila melanogaster* 377

CAIN, A. J., J. M. B. KING, and P. M. SHEPPARD, New data on the genetics of polymorphism in the snail *Cepaea nemoralis* L. . . . 393

HOLLANDER, W. F., J. H. D. BRYAN, and JOHN W. GOWEN, Pleiotropic effects of a mutant at the *P* locus from X-irradiated mice 413

FAHMY, O. G., and MYRTLE J. FAHMY, Cytogenetic analysis of the action of carcinogens and tumor inhibitors in *Drosophila melanogaster*. VII. Differential induction of visible to lethal mutations by related nitrogen mustards 419

CLARKE, C. A., and P. M. SHEPPARD, The genetics of *Papilio dardanus*, Brown. II. Races *dardanus, polytrophus, meseres,* and *tibullus* . . . 439

WEBER, E., The genetical analysis of characters with continuous variability on a Mendelian basis. II. Monohybrid segregation and linkage analysis 459

ZUCKER, LOIS M., Two-way selection for body size in rats, with observations on simultaneous changes in coat color pattern and hood size 467

ZUCKER, LOIS M., Model breeding experiment for two-way selection in small populations showing directional dominance 485

PTASHNE, MARK, The behavior of strong and weak centromeres at second anaphase of *Drosophila melanogaster* 499

PUBLISHED MONTHLY AT AUSTIN, TEXAS
BY GENETICS, INC.

(Date of issue, July 1, 1960)

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VOLUME 45 APRIL, 1960 NUMBER 4

GENETICS is a monthly journal issued in annual volumes of about 1000 pages each.

Subscription, $12.00 net a year for complete volumes (January–December). Foreign postage, $1.00 additional. Single copies, $1.50 each, postpaid.

As available, back volumes 1 through 44 may be had at $14.00 each and single issues of previous volumes at $2.50 per copy, postpaid. The Business Office will supply information on request as to volumes and numbers available.

Business Correspondence, including change of address, etc., should be addressed to GENETICS, INC., Business Office, Experimental Science Bldg. 122, University of Texas, Austin 12, Texas.

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Correspondence concerning editorial matters should be addressed to the EDITORS OF GENETICS, Experimental Science Building 122, University of Texas, Austin 12, Texas. 3579 should be sent to this address also.

Second-class mail privileges authorized at Austin, Texas.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Expansion of this issue to include extra papers has been made possible by a National Science Foundation Grant-G7194 which was given in part for support of publication of manuscript backlog. The Editors express appreciation for the support.

A PHYSIOLOGICAL STUDY OF THE VERMILION EYE COLOR MUTANTS OF DROSOPHILA MELANOGASTER[1]

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THE vermilion eye color mutant *Drosophila melanogaster* was one of the mutants used by BEADLE, EPHRUSSI, TATUM and their collaborators in their early attempts to interpret the biochemical effects of genes (Cf. Review by EPHRUSSI 1942). More recent studies on vermilion mutants (GREEN 1949, 1952, 1954) made it seem worthwhile to pursue further the study of the physiological effects of these mutants.

The sex-linked, recessive vermilion (v) eye color mutants of *D. melanogaster* are characterized phenotypically by a lack of brown eye pigment under the usual genetic and environmental conditions. GREEN (1952) distinguished two series of vermilion mutants by their differential interaction with a nonallelic X chromosome mutant, suppressor of vermilion (*su-v*). One group of v mutants approaches wild type in the production of brown eye pigment when combined with *su-v*. This series may be designated v^s, indicating that it is suppressible by *su-v*. The other series may be designated v^u, since it is unsuppressed by *su-v*. GREEN (1954) reported crossing over between a v^s allele (v^1) and a v^u allele (v^{36f}) indicating that they are pseudoallelic to each other. BARISH and FOX (1956) report that the v^u mutant, v^{48a}, is allelic to v^1 and pseudoallelic to v^{36f}.

STURTEVANT (1920) demonstrated by the use of gynandromorphs that the $v(v^1 = v^s)$ mutant was nonautonomous in development; i.e., flies which have genetically v eye tissue, but also contain v^+ tissue, may have phenotypically v^+ eyes. Transplantation of optic discs from v larvae into wild type larvae (EPHRUSSI and BEADLE 1935) confirmed the nonautonomous development of v with respect to wild type tissue. GREEN (1952) established developmental nonautonomy of the v^u mutants (v^{36f}, v^{51a}, v^{51b}, v^{51c}) with respect to wild type tissue in gynandromorphs. Studies by TATUM (1939), TATUM and HAAGEN-SMIT (1941), BUTENANDT, WEIDEL and BECKER (1940) and KIKKAWA (1941) established that genetically v flies will produce brown eye pigment if supplied with the tryptophan metabolite, kynurenine, either by injection or in the larval food.

GREEN (1949) found that large quantities of nonprotein tryptophan are accumulated by flies carrying v^1. Either kynurenine or its precursor, formylkynurenine, was shown to act as a precursor for brown eye pigment for both $v^1(v^s)$

[1] The material in this paper is taken from a thesis presented to the faculty of the University of California, Davis in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Second Printing 1966 / University of Texas Printing Division, Austin

and the v^u mutants, (v^{36f} and v^{51c}), by GREEN (1952). It has been established that in rat liver formylkynurenine is an intermediate in the conversion of tryptophan to kynurenine (KNOX and MEHLER 1950; MEHLER and KNOX 1950). It appears from the data of GREEN (1949, 1952) that in both classes of *v* mutants the block in brown eye pigment formation is prior to the formation of formylkynurenine, and, in the case of v^1, the block occurs between tryptophan and formylkynurenine. KNOX and MEHLER (1950) postulated two steps in the conversion of tryptophan to formylkynurenine. It appears that the two steps are catalyzed by the same enzyme in successive ferric-ferrous states (KNOX 1952, 1954) as a peroxidase and an oxidase. An intermediate between tryptophan and formylkynurenine has not been found (MEHLER 1955).

The evidence reported below supports the idea that both v^s and v^u mutants are blocked in the immediate utilization of tryptophan. Further differences between the two series of *v* mutants in their interaction with the suppressor mutant and with certain environmental situations are demonstrated. A brief report of these results has been made (SHAPARD 1954b).

MATERIALS AND METHODS

The mutants used in most of the experiments were the *D. melanogaster* mutants, $v^1(v^s)$, $v^{36f}(v^u)$ and su^2-*s*(*su*-*v*). Except where otherwise indicated these were combined with the autosomal recessive eye color mutant brown (*bw*) which blocks red eye pigment formation. The combination of the *bw* mutant with a *v* mutant results in the production of a nearly white eye, making it possible to determine more accurately the quantity of brown eye pigment which may be produced under experimental conditions. Three other *v* mutants: $v^2(v^s)$, $v^k(v^s)$ and $v^{51c}(v^u)$ were used in some experiments. An allele of su^2-*s*, su^{51c}-*v*, was used also. Cinnabar (*cn*), an autosomal recessive eye color mutant which fails to produce brown eye pigment and which accumulates kynurenine (KIKKAWA 1941) was used as a control in some tests. The sex-linked recessive mutants yellow (*y* and y^2), singed (sn^3), forked (*f*) and furrowed (*fw*) were present in some stocks. Most of the above mutants are described in BRIDGES and BREHME (1944). su^{51c}-*v* arose spontaneously in a y^2 *v f* stock at The University of Texas. v^k was obtained by AUERBACH from irradiated larvae; its suppression was shown by GREEN (personal communication). v^{51c} also was obtained in an X-ray experiment (GREEN 1952). No attempt was made to develop isogenic stocks. Certain special *D. melanogaster* stocks and stocks of other species will be described where appropriate.

Flies prepared by the following freeze-dry technique were used in biochemical and feeding experiments. Flies were raised on regular corn meal, molasses, agar, dry brewer's yeast medium seeded with live yeast (this will be referred to as regular medium) or on the same medium supplemented with 1 mgm DL-tryptophan/ml medium (referred to as tryptophan medium). Twenty females mated to 20 or more males were allowed to lay eggs for 24 hours or less on heavily yeasted bottles. After the parents were removed, "kleenex" soaked in a suspension of live

yeast was added to the bottles. Temperatures were maintained in a range of 23–25°C. Adult flies were collected 0–2 hours after emergence and the bottles from which they were collected were discarded 10–11 days after the beginning of the egg-laying period. The freshly collected flies were frozen in a bath of methyl cellosolve and solid CO_2. They were kept in the bath at least one hour, transferred to a vacuum desiccator containing "drierite" ($CaSO_4$) and dried *in vacuo* for 24 hours or more, then stored in the vacuum desiccator until used.

Analysis for nonprotein tryptophan was performed on weighed samples of dried flies which had been pulverized in an agate mortar. Protein was precipitated with a ten percent solution of trichloracetic acid. The mixture was filtered, and tryptophan was determined on the filtrate by the HORN and JONES (1945) modification of the MAY-ROSE *p*-dimethylaminobenzaldehyde method at a wave length of 600 millimicrons on a Coleman Junior spectrophotometer, Model 6a. Tryptophan in this paper always refers to nonprotein tryptophan. A standard curve was made with L-tryptophan in aqueous solution. The range from 0.02–0.04 mgm tryptophan is most sensitive. Where it is indicated that a determination is outside the range of accuracy of the test, the amount of material used had a quantity of tryptophan outside the range 0.02–0.04 mgm but within the range 0.00–0.06 mgm for which an optical density *vs.* L-tryptophan curve was determined. Paper chromatography provides further evidence that the substance which is accumulated by *v* flies is indeed tryptophan. It does not separate from L-tryptophan when chromatographed by either ascending or circular techniques with 4 butanol:5 water:1 acetic acid solvent and stains the purple of tryptophan when stained with Erlich's reagent prepared by the method of SMITH (1953).

EXPERIMENTAL RESULTS

Accumulation of nonprotein tryptophan by v^s *and* v^u *mutants*

Tryptophan is high in both v^s and v^u mutants as compared to v^+ flies (Tables 1 and 2). An increased accumulation of tryptophan by both classes of mutants was observed in flies raised on the medium supplemented with tryptophan. The quantity of tryptophan in v^+ flies does not appear to be affected by the amount of tryptophan in the medium. A comparison with GREEN's 1949 report of 0.236 mgm tryptophan/gm dried flies for v^+; *bw* and 1.389 mgm/gm for $v(v^1)$ suggests that his medium may have had a higher tryptophan content.

Determinations made on flies raised at the same time, and under similar culture conditions (Table 1), indicate that v^1; *bw* flies accumulate slightly less tryptophan than do flies of the v^{36f}; *bw* genotype. This might be expected since the v^{36f} mutant and other v^u mutants combined with the *bw* mutant actually have white eyes. The v^1 mutant and other v^s mutants when combined with the *bw* mutant produce eyes with a slight brownish tinge indicating that the block in the production of brown eye pigment is not complete. However, except in experiment "j" on regular medium, this difference is small. The data with respect to flies of the genotypes v^2 *fw*(v^s) and *y sn*^{3} v^{51c}; *bw* (v^u) (Table 2) do not allow a generalization that v^s mutants accumulate less tryptophan than do v^u mutants. The

and five test larvae (65–79 hours after egg laying) were placed on the medium; all tests were run in duplicate. The control medium was the same food with no pupae added. The emerging *cn bw* flies all had white eyes. Flies homozygous for v^1, v^{36f} or v^{51c} mutant alleles all produced brown eye pigment when fed *cn* pupae but had white eyes when raised on the control medium or when fed the same or any other *v* mutant pupae. Similar results were obtained by feeding lyophilized adults to test larvae. Two hundred and fifty mgm of dried flies which had been ground in an agate mortar were added to 2 ml of the yeast suspension. Transfer of larvae to the experimental food was made 58–71 hours after egg laying. Flies of the *cn bw* genotype were used as controls, and v^1 and v^{36f} mutants were tested. Both v^1; *bw* and v^{36f}; *bw* flies developed brown eye pigment if the larvae were fed on *cn bw* adults, but not if they were fed on either type of *v* adults. Thus feeding either pupae or adults gives no evidence that either class of *v* mutants accumulates a substance which the other class can utilize in the production of brown eye pigment. Lyophilized su^2-*s* v^{36f}; *bw* adults fed to v^1; *bw* larvae appeared to cause the production of a small amount of brown eye pigment; however, the effect was so slight as to be of doubtful significance.

Feeding tryptophan analogs: It remains possible that if the immediate product of trytophan metabolism could be obtained from some other source, either biological or chemical, it might behave as a precursor to brown eye pigment if fed to *v* larvae of one or both types. Dr. Bernhard Witkop (United States Public Health Service) has kindly supplied us with a series of tryptophan analogs (DL-2-phenyltryptophan, DL-2-carbethoxytryptophan, 5(OH)tryptophan, 7(OH)-tryptophan), none of which was thought to be the actual immediate product of tryptophan metabolism, but all of which were tested for possible activity as a precursor of brown eye pigment in the mutant flies. The methods used were those which Green (1952) used for feeding kynurenine and formylkynurenine. Both kynurenine and formylkynurenine produce a color intermediate between the *v*; *bw* phenotype and the v^+; *bw* phenotype when fed to v^{36f}; *bw* or v^1; *bw* flies at a concentration of 1 mgm/2 ml medium (Green 1952). None of the tryptophan analogs tested at a concentration of 1.0 − 1.2 mgm/2 ml medium caused the production of brown eye pigment in the flies tested: (v^1; *bw*), (v^{36f}; *bw*) and (*cn bw*). Feeding of tryptophan is reported in a later section.

Accumulation of tryptophan by v^s *and* v^u *mutants combined with the* su-v *mutant*

Tryptophan accumulation by the two classes of *v* mutants in combination with the suppressor mutant was determined in order to ascertain whether the suppressor differentiated the two classes on this level as well as on the level of eye color phenotype. Green (1949) has shown that the *su-v* mutant reduces the accumulation of tryptophan by the v^1 mutant. Tryptophan accumulation in the two mutant classes and the heterozygotes between them in combination with the homozygous or heterozygous *su-v* genes is recorded in Tables 3 and 4.

The mutant classes v^s and v^u are clearly differentiated by the quantity of tryptophan which they accumulate when combined with the homozygous *su-v*

TABLE 3

Nonprotein tryptophan composition of adult flies of v *mutants combined with homozygous or heterozygous* su-v *mutant*

	Regular medium				Tryptophan supplement				
			mgm tryptophan*				mgm tryptophan		
Genotype	Sex	Ex.	Avg.	Range	Sex	Ex.	Avg.	Range	Eye color
su²-s v¹; bw	♀♀	j	0.405(2)	0.395–0.414	♀♀	i	0.469(2)	0.460–0.477	3.0
	♂♀	a	0.372(2)	0.368–0.372					
su²-s v²: bw	♂♀	e	0.368(3)	0.349–0.380	♀♀	i	0.381(2)	0.367–0.395	3.5
su²-s v¹/su²-s v²; bw	♀♀	e	0.339(2)	0.326–0.352					
v¹/su²-s v¹; bw	♀♀	j	0.685(2)	0.685–0.685	♀♀	i	0.776(2)	0.759–0.793	0.7
v¹/su²-s v²; bw	♀♀	e	0.675(2)	0.655–0.694					
v² fw/su²-s v¹					♀♀	i	0.855(2)	0.805–0.904	. .
su²-s v³⁶ᶠ; bw	♀♀	j	0.773(2)	0.757–0.788	♀♀	i	0.884(2)	0.852–0.915	0.0
	♂♀	e	0.902(2)	0.890–0.914					0.0
su²-s v⁵¹ᶜ f; bw	♂♂	a	0.778(1)	. . . . .					0.0
	♂♂	b	0.762(1)	. . . . .					0.0
su²-s v⁵¹ᶜ					♀♀	i	0.822(2)	0.807–0.836	. .
su²-s v⁵¹ᶜ/su²-s v³⁶ᶠ	♀♀	e	0.813(2)	0.762–0.863					. .
v³⁶ᶠ/su²-s v³⁶ᶠ; bw	♀♀	j	0.788(2)	0.784–0.792	♀♀	i	0.985(2)	0.975–0.994	0.0

* See footnotes of Table 1 for abbreviations.

TABLE 4

Nonprotein tryptophan composition of adult v^s/v^u *heterozygous females with homozygous or heterozygous* su-v

	Regular medium			Tryptophan supplement			
	mgm tryptophan*			mgm tryptophan			
Genotype	Ex.	Avg.	Range	Ex.	Avg.	Range	Eye color
su²-s v¹/su²-s v³⁶ᶠ; bw	j	0.574(2)	0.572–0.575	i	0.598(2)	0.595–0.601	1.5
	e	0.485(2)	0.478–0.492				1.5
su²-s v¹/su²-s v⁵¹ᶜ				i	0.701(2)	0.672–0.730	. .
su²-s v³⁶ᶠ/su²-s v²; bw				i	0.717(2)	0.714–0.720	1.5
v¹/su²-s v³⁶ᶠ; bw	j	0.753(2)	0.752–0.754	i	0.910(2)	0.900–0.920	0.0⁺
v³⁶ᶠ/su²-s v¹; bw	j	0.746(2)	0.730–0.762	i	0.914(2)	0.909–0.918	0.0⁺–0.1
y sn³ v⁵¹ᶜ/su²-s v¹; bw				i	0.838(2)	0.818–0.857	0.0⁺
v³⁶ᶠ/su²-s v²; bw				i	0.857(2)	0.839–0.875	0.0⁺
v¹/su²-s v⁵¹ᶜ; bw				i	0.947(2)	0.927–0.966	0.0⁺
v² fw/su²-s v³⁶ᶠ; bw/+				i	0.916(2)	0.872–0.960	. .

* See footnotes of Table 1 for abbreviations.

mutant (Table 3). Both v^1 and v^2, the v^s mutants tested, accumulate much less tryptophan when combined with the *su-v* mutant (Cf. Tables 1 and 2 with Table 3). However, v^u mutants (v^{36f} and v^{51c}) continue to accumulate a high level of tryptophan in the presence of the homozygous suppressor (Table 3). Where there are strictly comparable data, as in experiments j and i, there appears to be a little less tryptophan in flies of the genotype *su²-s* v^{36f}*; bw* than in

v^{36f}; *bw* flies (Cf. Tables 1 and 3). This could be indicative of a quantitative rather than a qualitative difference between v^s and v^u mutants in their interaction with the suppressor, or it may indicate an effect of the suppressor on tryptophan utilization in addition to its interaction with the v^s mutants.

It is clear from Table 3 that the suppressor behaves as an incompletely dominant gene in its effects on the v^s mutant, both with regard to eye color and to accumulation of tryptophan. Also, the homozygous suppressor mutant has a clear effect on the heterozygote v^s/v^u (Table 4). Thus the combined effect of the *su-v* mutant with the v^s mutants depends both on the dosage of v^s alleles and on the dosage of the *su-v* alleles. The data from experiment i (Table 4) indicate a slight effect of the suppressor mutant in heterozygous condition on v^s/v^u flies as might be expected from the dosage relationships.

Further information on dosage relationships was obtained through the use of a deficiency for the suppressor. Since only a small number of flies was obtained in this manner, only the effect on pigment production was considered. If flies are constituted which carry the X chromosome of T(1;2)*Bld* (BRIDGES and BREHME 1944) and a normal second chromosome, they are deficient for the tip of the X chromosome including the loci of *y* and *su-v*. The alleles v^1 and v^{36f} were each introduced into the X chromosome of T(1;2)*Bld*, and flies of the following genotypes were constituted. The number of flies obtained and their average eye color (Based on the scale of TATUM and BEADLE 1939) are also indicated.

Genotype	Number flies	Average color
$\frac{\text{X from T(1;2)}Bld,\ v^1\ ;\ bw}{y^2\ su^{51c}\text{-}v\ v^1\ f}$	23	3.1
$\frac{\text{X from T(1;2)}Bld,\ v^1\ ;\ bw}{y^2\ su^{51c}\text{-}v\ v^{36f}}$	96	1.2
$\frac{\text{X from T(1;2)}\ Bld,\ v^{36f}\ ;\ bw}{y^2\ su^{51c}\text{-}v\ v^{36f}}$	17	1.7
$\frac{\text{X from T(1;2)}\ Bld,\ v^{36f}\ ;\ bw}{y^2\ su^{51c}\text{-}v\ v^{36f}}$	38	0.0

These data indicate that a deficiency for suppressor in combination with one suppressor gene gives essentially the same eye color effect as does the homozygous suppressor mutant (Cf. Tables 3 and 4). Thus, it appears that the absence of su^+-*v* allows the production of brown eye pigment in a v^s mutant.

Starvation

Another technique used to differentiate the v^s and v^u mutants is the "starvation effect." BEADLE, TATUM and CLANCY (1939) observed that if *v; bw* larvae were removed from complete medium to a medium containing only 0.5–1.0 percent dry brewer's yeast at 60–70 hours after egg laying, the level of brown eye pigment in the emerging adults was increased over that of the controls on

complete food. TATUM and BEADLE (1939) reported that the most effective and efficient method for the production of the "starvation effect" was to transfer sterile eggs to a medium of 0.5 percent dry brewer's yeast in 1.5 percent agar and to allow the larvae to develop at 25°C. Under these conditions, there was a prolongation of larval life such that the time from egg laying to emergence of the adult was increased from the normal nine days to 11–13 days. A color of 2.5–3.5 on a scale where *v; bw* = 0, *bw* = 5 was observed in the emerging *v; bw* flies.

The "starvation" medium used in experiments to be reported here was 0.5 percent yeast in 1.5 percent agar; the control medium was 12 percent yeast in 1.5 percent agar. Flies were allowed to oviposit on freshly yeasted vials of regular medium. Eggs were washed with distilled water and 70 percent alcohol; larvae, with distilled water. Ten eggs or larvae (52–66 hours after egg laying) were placed in each vial containing 2 ml of "starvation" or control medium. An increase of 2–4 days in the time from egg laying to emergence of adults was observed on the "starvation" medium as compared to the control medium whether eggs or larvae were used. At least two vials (in most cases three) were made of each genotype on each medium. Males and females of (v^1; *bw*), (v^{36f}; *bw*) and (y sn^3 v^{51c}; *bw*) and heterozygous females (v^1/v^{36f}; *bw*), (y sn^3 v^{51c}/v^1; *bw*) and (v^{36f}/y sn^3 v^{51c}; *bw*) were tested both as eggs and larvae. *bw* and *cn bw* were also used in the experiments using eggs. Visual comparisons were made of eye colors which were classified on the scale of TATUM and BEADLE (1939). The eye color of v^1; *bw* was classified 0.0+ on the control medium and 2.0–3.5 on "starvation" medium. No difference was observed between "starvation" and control flies of any of the other homozygotes. The v^1 mutant is then distinguished from the v^{36f} and v^{51c} mutants by the environmental condition of partial starvation of larvae as well as by the genetic suppressor mutant. There appeared to be a very slight effect of "starvation" on the v^1/v^{36f}; *bw* females but it was so small as to be of doubtful significance, and the other heterozygotes did not show any change in eye color in response to "starvation." BARISH and FOX (1956) report that v^{48a} (a v^u mutant) is not affected by "starvation." We recently investigated another v^s mutant, v^k, and it responded in the same manner as v^1; i.e., it developed an eye color of 2.0–3.0 on "starvation" medium though it was almost white on the control medium. Flies of the genotype ras^2 v^1 v^{36f} *m f* (obtained from M. M. GREEN who had recovered it from a cross-over between v^1 and v^{36f} in an attached-X female, GREEN 1954) were also tested, and no evidence for a starvation effect was found for this combination of vermilion alleles.

The effect of larval "starvation" on tryptophan accumulation by the mutants was also studied. Attempts were made to raise the flies in bottles in which at least 300 eggs had been placed on 50 ml "starvation" medium. However, though the number of eggs per unit of food was greater than that used in the vials (ten eggs/2 ml "starvation" medium), and the flies were not collected at less than 13 days after egg laying, the brown eye pigment of the v^1; *bw* flies was only

slightly increased (the color was about 1.0 as opposed to 2.5–3.5 in the experiments run in vials). The genotypes studied were: (*bw*), (v^1; *bw*), (v^{36f}; *bw*) and (v^1/v^{36f}; *bw*). The tryptophan in mgm/gm dried flies is essentially the same for all genotypes as in experiments involving regular medium (Cf. Tables 5 and 1). Also it is clear that either on a mgm/gm dried flies basis or on a mgm/1000 flies basis, the tryptophan accumulation is very nearly the same in v^1; *bw* as in v^{36f}; *bw* flies. It was not clear from these experiments whether starvation failed to differentiate between v^1 and v^{36f} with regard to tryptophan accumulation, or whether we had failed to attain "starvation" conditions when raising the flies in bottles rather than vials.

Therefore, a series of new experiments was begun, culturing the flies in vials. These were performed in a different laboratory (during the tenure of a Postdoctoral fellowship from the National Institutes of Health at The University of Texas, Department of Zoology), and for reasons still not clear, the adults would not emerge on the "starvation medium" which had been used in the previous experiments (and which was the same as that used by TATUM and BEADLE (1939)). We therefore derived a different medium containing 0.5 gm Fleischmann dry brewer's yeast and 0.5 gm Difco Bacto yeast extract (water soluble fraction of autolyzed yeast) in 1.5 percent agar. This was supplemented with streptomycin and propionic acid in the same manner as the original medium. This medium gave a clear-cut starvation effect (color of v^1; *bw* adults emerging on it was 2.5–3.0), and a high percentage of adults emerged. A control medium was used consisting of the same ingredients plus two percent sucrose. TATUM and BEADLE (1939) had shown that the addition of two percent sucrose to the "starvation" medium resulted in the same delay in larval development but completely inhibited the effect on eye pigment production. The sterilization procedure used in these experiments was to transfer eggs from vials of regular medium (not seeded with live yeast) to sterile water in watch glasses lined with filter paper. The agar was washed from the eggs and they were then transferred to a mercuric chloride solution (0.5 gm $HgCl_2$, 6.5 gm NaCl, 1.25 ml conc. HCl, 250 ml ethyl alcohol brought up to 1000 ml with sterile H_2O) contained in sterile watch glasses in petri dishes. The eggs remained in the $HgCl_2$ solution 20–30

TABLE 5

Nonprotein tryptophan composition of D. melanogaster *mutants raised on "starvation" medium in half pint bottles*

		Tryptophan		Tryptophan	
		mgm/gm dried flies		mgm/1000 flies	
Genotype	Sex	Avg	Range	(Avg.)	Eye color
bw	♂ ♀	0.236(2)	0.214–0.258	0.047(2)	5.0
v^1; *bw*	♂ ♀	0.738(3)	0.709–0.769	0.146(3)	1.0
v^{36f}; *bw*	♂ ♀	0.759(2)	0.757–0.760	0.155(2)	0.0
v^1/v^{36f}; *bw*	♀ ♀	0.831(2)	0.829–0.832	0.154(2)	0.0*

* See Table 1 for abbreviations.

minutes; the solution was then siphoned off and the eggs washed with two or three changes of 70 percent ethyl alcohol. The eggs were left in the last change of alcohol for 20–30 minutes, then transferred in lots of 20 by means of a sterile needle to "starvation" or "sucrose" vials containing 4 ml of medium. The sucrose did not completely inhibit eye pigment formation in v^1; *bw* under these circumstances; however, the color was around 1.0 while it was 2.5–3.0 on the "starvation" medium. The amount of material which we were able to get under these circumstances allowed only a small number of assays (Table 6), but it seems clear that the effect of "starvation" must be quite different from that of the genetic suppressor since there is no evidence for a decrease in tryptophan accumulation when brown pigment is produced. The results on sucrose medium were based on quite small samples, and the significance of the apparent reduction in tryptophan accumulation by both mutants is not clear.

Feeding tryptophan

VALADERES and CHARCONNET (1950) report that by varying the quantity of tryptophan in a synthetic medium, the quantity of brown eye pigment produced by *D. melanogaster* may be altered. Genetically v^+ eyes may be caused to approach the v phenotype by limiting the amount of tryptophan, while increasing tryptophan in the medium causes genetically v eyes to approach v^+ eyes in phenotype. An attempt was made to repeat the latter results in order that they might possibly be used as one method of differentiating the two classes of v mutants. A synthetic medium was not used in our experiments. A control medium was used consisting of 12 percent dry brewer's yeast in a 1.5 percent agar base. Mold and bacterial growth were inhibited by 0.01 ml propionic acid and 25 gamma streptomycin/ml medium. For experimental purposes the 12 percent yeast medium was supplemented with 4 mgm, 2 mgm, or 1 mgm DL-tryptophan or 2 mgm or 1 mgm L-tryptophan/ml medium. Eggs as well as larvae of various ages of (*cn bw*), (v^1; *bw*) and (v^{36l}; *bw*) genotypes were transferred to the test and control media (ten eggs or larvae/vial containing 2 ml medium). No effect on brown eye pigment in flies of any of these genotypes was found at any of the concentrations tested. VALADERES and CHARCONNET

TABLE 6

Nonprotein tryptophan composition of D. melanogaster *mutants raised on "starvation" and "sucrose" media (Expts. performed at Univ. of Texas, see text)*

	Starvation medium			Sucrose medium		
	mgm tryptophan*			mgm tryptophan		
Genotype	Avg.	Range	Eye color	Avg.	Range	Eye color
v^+; *bw*	0.204(1)	.....	5.0	0.204(1)	.....	5.0
v^1; *bw*	0.878(2)	0.815–0.941	2.5–3.0	0.563(1)	.....	0.8–1.5
v^{36l}; *bw*	1.090(2)	1.075–1.104	0.0	0.729(2)	0.690–0.768	0.0

* See footnote of Table 1 for abbreviations.

report positive effects on *v*; *bw* flies at concentrations of 1.0–1.5 mgm L-tryptophan/ml medium.

The results of VALADERES and CHARCONNET might be expected as a "starvation effect." They use the medium of "SCHULTZ *et coll.*" (presumably SCHULZ, ST. LAWRENCE and NEWMEYER 1946) which causes a lengthening of larval life (approximately 8–9 days from egg laying to pupation) of about the same amount characteristic of the conditions producing the "starvation effect."

Comparison of homologous mutants in other species

The sex-linked mutant vermilion (*v*) of *D. simulans* accumulates tryptophan at a level much higher than do the wild type individuals of the same species (Table 7) and it does not respond to conditions of larval "starvation" by producing brown eye pigment. The evidence points to a tentative homology of the *v* mutant of *D. simulans* to the v^u mutants of *D. melanogaster*.

Two mutants of *D. virilis* which fail to produce brown eye pigment under normal conditions have characteristics similar to the *v* mutants of *D. melanogaster*. These are the sex-linked vermilion mutant (*v*) and the autosomal mutant cardinal (*cd*). Optic discs of both *v* and *cd* develop nonautonomously when implanted into wild type or scarlet larvae (PRICE 1949). The mutant scarlet is apparently homologous to the *cn* mutant of *D. melanogaster* (PRICE 1949; GREEN 1949). Both *v* and *cd* mutants produce brown eye pigment when fed kynurenine (PRICE 1949; GREEN 1952) or formylkynurenine (GREEN 1952). GREEN (1949) has shown that the *v* mutant of *D. virilis* accumulates about four times as much nonprotein tryptophan as do wild type or eosinoid flies. The mutant eosinoid (*es*) appears to be homologous to the *bw* mutant of *D. melanogaster* (MORI 1937).

The mutants *v* and *cd* of *D. virilis* were combined with the *es* mutant in the studies to be reported here. It may be seen from Table 7 that the *cd* mutant, as well as the *v* mutant, accumulates a high level of tryptophan. The two mutants were also compared in regard to their response to larval "starvation." The *cd* mutant produces more brown eye pigment on "starvation" medium than on the control medium, but no response of the *v* mutant was observed. GREEN (1955) suggests that *v* of *D. virilis* may be homologous to v^u of *D. melanogaster* and *cd* of *D. virilis* to v^s of *D. melanogaster*. He presents evidence indicating that *cd* will respond to the suppressor of vermilion from *D. melanogaster* when eye discs of *cd* are transplanted into hosts carrying the suppressor and an unsuppressible *v* mutant. The *v* mutant of *D. virilis* did not respond to the *D. melanogaster* suppressor.

The accumulation of tryptophan in the meal moth, *Ephestia kuhniella*, (Table 7) is also of interest in regard to interspecific gene homologies. Our value for nonprotein tryptophan in wild type moths can only be considered to be very low since such a value was outside the range of accuracy of the test. However, the values for the *a/a* mutant, which fails to produce brown eye pigment, are within the range of accuracy, and it is clear that the *a/a* individuals accumulate

TABLE 7

Nonprotein tryptophan composition and starvation response of mutants of other Drosophila species and of Ephestia kuhniella

Species	Genotype	mgm tryptophan* Avg.	mgm tryptophan* Range	Starvation response
D. simulans	+	0.304(2)	0.290–0.318	..
	v	0.783(3)	0.705–0.823	0
D. virilis	*es*	0.143†(2)	0.133–0.143	..
	v^{40d}; *es*	0.722(3)	0.650–0.961	0
	cd; *es*	0.632(3)	0.612–0.648	+
	v^{40d}/+; *cd*/+	0.194(2)	0.191–0.196	..
Ephestia kuhniella	+	0.067(1)†	.....	..
	a/*a*	0.346(2)	0.329–0.364	0(CASPARI 1943)

* See footnotes of Table 1 for abbreviations.
† Outside range of accuracy of test.
The lyophilized adults of Ephestia were kindly provided by DR. M. M. GREEN.

a much higher level of nonprotein tryptophan than do wild type moths. CASPARI and RICHARDS (1948) report a value of 0.04 mgm nonprotein tryptophan/gm wet wt. larvae for wild type and 0.10 mgm/gm for *a*/*a* larvae. CHEN and KUHN (1956), using paper chromatography, detected nonprotein tryptophan in several developmental stages of *a* mutant moths, but not in white eye(*wa*) mutants or wild type moths studied at the same developmental stages. Protein tryptophan is also higher in *a* mutants than in wild type (CASPARI 1946; CASPARI and RICHARDS 1948; BUTENANDT and ALBRECHT 1952); however, this may be a secondary effect of nonprotein tryptophan accumulation. It seems probable that the primary actions of a^+ and *a* of Ephestia are homologous to those of v^+ and *v* of *D. melanogaster*. If they are homologous, it is interesting to speculate whether *a* is a v^u or v^s type mutant. CASPARI (1943) found that the *a* mutant did not produce brown eye pigment when the larvae were subjected to starvation conditions, possibly indicating an homology to the v^u mutant. The *a* mutant has recently been studied by KUHN and EGELHAAF (1958), and they were able to show that it lacked the ability to oxidize tryptophan *in vitro*. They were able to demonstrate the ability of wild type tissues to carry out this process. This is of particular interest since it is the first demonstration of the *in vitro* oxidation of tryptophan in insects. It may make possible further studies of the enzyme in Drosophila. Many workers have previously attempted to obtain such enzyme activity in wild type Drosophila but have failed up to now.

DISCUSSION

The results reported here establish certain similarities and differences between v^s and v^u mutants which may be considered in formulating a tentative theory of their mode of action and their interaction with the suppressor mutant.

Phenotypically both v^s and v^u flies differ from wild type individuals in their

failure to produce brown eye pigment. Both develop nonautonomously with respect to wild type tissue in gynandromorphs (STURTEVANT 1920; GREEN 1952) and produce brown eye pigment when fed kynurenine (BUTENANDT, WEIDEL, and BECKER 1940; GREEN 1952) or formylkynurenine (GREEN 1952). Mutants of both classes accumulate tryptophan at a level several times that of v^+ flies. A trace of brown eye pigment is produced by v^s flies, and it may be that they accumulate slightly less tryptophan than do v^u flies. There is no evidence that either class of v mutants accumulates a diffusible substance which the other class of mutants can utilize in the production of brown eye pigment. Both cause a block in the conversion of tryptophan to formylkynurenine, and the evidence so far accumulated is compatible with the idea that both mutants block the tryptophan peroxidase-oxidase system in the immediate conversion of tryptophan to the intermediate compound.

Mutants of the two classes, v^s and v^u, differ in their response to a nonallelic suppressor gene. Flies of the v^s type produce brown eye pigment in the presence of the suppressor mutant (GREEN 1952), and there is a concomitant decrease in accumulation of non-protein tryptophan (GREEN 1949). However, the suppressor mutant causes no eye color change in v^u mutants (GREEN 1952) and has little, if any, effect on accumulation of tryptophan by v^u mutants. Flies heterozygous for the suppressor and a deficiency for suppressor produce eye pigment in the same amount as those homozygous for suppressor. The "starvation effect" also differentiates the two classes of v mutants; v^s (v^l and v^k) flies produce brown eye pigment under conditions of partial starvation of larvae, while flies of the v^u (v^{36f}, v^{48a}, v^{51c}) type fail to produce brown eye pigment under the same conditions. When v^{36f} and v^l are on the same chromosome, both the suppressor (GREEN 1954) and "starvation" fail to affect the production of brown eye pigment.

The suppressor mutant which interacts with the v^s mutants to allow the production of brown eye pigment also interacts with several dissimilar nonallelic mutants causing them to approach wild type in phenotype (GREEN 1954). One such mutant is purple (*pr*) which causes a reduction in red eye pigment and an increase in brown eye pigment (NOLTE 1955), develops autonomously with respect to wild type tissue (BEADLE and EPHRUSSI 1936), does not accumulate nonprotein tryptophan and does not approach wild type in phenotype when subjected to larval "starvation" (SHAPARD 1954a). The same suppressor mutant also suppresses the mutant effect of sable (BRIDGES 1932), a temperature sensitive sex-linked mutant which causes abnormally black body and is nonautonomous (E. B. LEWIS personal communication). H. W. LEWIS (1955) and H. W. LEWIS and H. S. LEWIS (1958) have shown that both the sable mutant and suppressor of sable affect tyrosinase activity. Another mutant which is suppressed is the autosomal mutant speck (*sp*) (BRIDGES and BREHME 1944; GREEN 1954) which causes a darkening of body color and a black speck at the base of the wings.

The actual effect of the v^s and v^u mutant genes and of the suppressor mutant on the tryptophan peroxidase-oxidase system can be determined only through a study of these enzymes in wild type individuals and in the v mutant with and

without the suppressor mutant. It has not been possible so far to obtain an active tryptophan oxidizing preparation from Drosophila, so these studies have not been made. KUHN and EGELHAAF (1958) have obtained a tryptophan oxidizing enzyme from Ephestia and have demonstrated that the *a* mutant of Ephestia lacks tryptophan oxidizing activity *in vitro*. GLASSMAN (1956) demonstrated activity for kynurenine formamidase (the enzyme which converts N′-formylkynurenine to kynurenine) in cell free extracts of both wild type and various mutant lines of *D. melanogaster* including both v^s and v^u mutant types. This is further evidence that the enzyme system affected by the *v* mutants must be the one which affects the conversion of tryptophan to formylkynurenine. GLASSMAN and many other workers including the present author have failed to obtain tryptophan oxidizing activity in *in vitro* preparations of Drosophila tissues. It is hoped that the techniques of KUHN and EGELHAAF (1958) may make it possible to obtain this activity in Drosophila and thus to investigate the effects of these mutants on this enzyme system.

We can say little about the action of the v^u mutants except that the block in tryptophan metabolism is so effective that we have not found a means of overcoming it (we can cause the production of brown eye pigment by feeding tryptophan metabolites, but presumably this does not overcome the block in tryptophan oxidation).

However, with regard to v^s mutants and the suppressor, we have some basis for speculation. The v^s mutants appear to have an incomplete block since a small amount of brown eye pigment is produced. This block may be partially overcome in at least two ways: (1) genetic suppression which causes the production of brown eye pigment and a reduction in the accumulation of tryptophan and (2) larval "starvation" which causes a production of brown eye pigment and of v^+ hormone (BEADLE, TATUM, and CLANCY 1938); therefore, it presumably acts on the production of kynurenine, but does not noticeably reduce the accumulation of tryptophan. At least one other mutant (*pr*) which is affected by the suppressor is not affected by starvation under the same conditions as those under which v^s is caused to produce brown eye pigment. A deficiency for the suppressor gene appears to be as effective as a mutation to the suppressor allele. This would indicate that a substance produced when su^+-*v* is present interacts in some way with a substance produced in the presence of v^s to block tryptophan metabolism. In the absence of this su^+-*v* substance, tryptophan is oxidized though at a lower rate than in wild type flies. Apparently, the heterozygote, su^+-*v*/*su*-*v*, produces less of this substance since such flies are intermediate between the two homozygotes. The evidence, so far, indicates that starvation may affect a later step in the reaction sequence, perhaps blocking a side reaction, thus allowing more of any tryptophan metabolites that are produced to be diverted to the production of brown eye pigment.

SUMMARY

The suppressed (v^s) and unsuppressed (v^u) vermilion eye color mutants of *Drosophila melanogaster* have been further studied and found to differ in several

physiological and biochemical characteristics. Both v^s and v^w mutants fail to produce brown eye pigment under normal genetic and environmental conditions, and both accumulate nonprotein tryptophan. Feeding tests, gynandromorphs between the two mutants, and transplantation experiments fail to indicate any difference between the two mutant classes.

The semidominant nonallelic suppressor gene (*su-v*) suppresses both the eye color phenotype and the accumulation of nonprotein tryptophan in v^s mutants but has no apparent effect on either trait in v^w mutants. The eye color effect of the v^s mutants may also be overcome by partial starvation of larvae; nonprotein tryptophan accumulation does not appear to be affected by "starvation." There is no observable effect of "starvation" on the v^w mutants.

Analogous mutants in other species of Drosophila and in *Ephestia kuhniella* are shown to correspond in their physiological characteristics to the two classes of *v* mutants of *D. melanogaster*.

The significance of these results for an interpretation of the action of the *v* mutants and of the suppressor is discussed. It is concluded that both v^s and v^w mutants probably block the conversion of tryptophan to formylkynurenine, but that the nature of the block produced by the two classes of mutants is different. The suppressor probably has a "negative" effect, i.e., the su^+-*v* substance is not produced and v^s flies are able to metabolize some tryptophan in the absence of this substance. Starvation may affect a later step in the reaction sequence.

ACKNOWLEDGMENTS

This work was done under the guidance of Dr. M. M. Green to whom the author is grateful for his interest and advice. Parts of the work were completed or verified during the tenure of a National Cancer Institute, National Institutes of Health, Postdoctoral Fellowship held at The University of Texas, Department of Zoology, and under National Science Foundation Grant G-3842 at Smith College.

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THE GENETIC BASIS OF NATURAL VARIATION. II. ANALYSIS OF A POLYGENIC SYSTEM IN DROSOPHILA MELANOGASTER[1]

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Received July 13, 1959

MUCH natural variation has a polygenic basis. An investigation of such natural variation leads one to the ground lying between the analysis of single-locus variation and the analysis of complex phenotypes without investigating specific genes.

Several attempts have been made to carry the techniques of classical genetic analysis into the complex realm of naturally occurring polygenes (WADDINGTON 1953; BARIGOZZI and DI PASQUALE 1956; MILKMAN 1956, 1958, 1960a, b). The work reported here is a continuation of such an effort.

The crossveinless (*cve*) complex has been described (MILKMAN 1960b) as a group of common, widespread, naturally occurring polygenes, distributed over the major chromosomes of *D. melanogaster*, and interacting to affect the ability of the developing animal to make posterior crossveins. This is in agreement with the various conclusions reached as a result of previous or contemporary studies of the *cve* genes (WADDINGTON 1953; MOHLER 1955; BATEMAN 1955 and 1956). These attributes of the *cve* genes, together with their susceptibility to various lines of investigation, make them a likely choice for detailed analysis.

MATERIALS AND METHODS

General materials and methods, as well as the results obtained from the basic analysis of the crossveinless complex, may be found in the first paper of this series (MILKMAN 1960b). In brief, a true-breeding, polygenic crossveinless strain was obtained from wild Massachusetts flies by selection. The criterion for selection was, in the early stages, susceptibility to production of crossveinless phenocopies, a trait shown to be one aspect of low crossvein making ability (MILKMAN 1960b). The *cve* genes cause defects of the posterior crossveins of the wings, and this effect is more pronounced at 18°C than at 25°C. It was possible to rate the phenotype quantitatively in terms of the crossvein fraction missing. Each wing can be rated from 0 (normal; no sixths missing) to six (crossvein

[1] Most of this paper is taken from a thesis in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the subject of Biology at Harvard University.

[2] The major part of the research described was supported by a predoctoral fellowship of the National Science Foundation for the academic year 1955–56. Current work referred to is supported by grants from the Horace H. Rackham Fund (R-420) and the Michigan Memorial-Phoenix Project, both of The University of Michigan.

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completely absent; six sixths missing). A fly may therefore be rated from 0 to 12, by adding the individual wing ratings.

With the findings that the *cve* genes are on all three major chromosomes and that they act additively in lowering posterior crossvein making ability, it was desirable to push the investigation towards a more detailed characterization of individual genes. Two approaches were used: one involved the relationship of known mutant genes to the *cve* system; the other made use of recombination experiments to localize the genes as precisely as possible.

EXPERIMENTAL RESULTS

Interaction with known genes that cause defective crossveins and with other suspect genes: There are a number of single mutant genes which interfere with posterior crossvein formation, some of which have characteristic effects on the posterior crossvein which distinguish them from others. With regard to these genes, three possibilities present themselves: 1. That the *cve* complex is a balanced system, heterozygous for some of these factors. 2. That alleles of these genes are the components of the complex, alleles which have no visible effects by themselves but which do in combination with other genes. 3. That these genes are not a part of the complex.

The first possibility may be discarded on the basis of evidence against the *cve* complex being a balanced system, namely, that crossover data and cytological examination fail to indicate the presence of heterozygous inversions.

The other possibilities may be tested by a combination of experiments designed to measure similarity between any gene and the components of the *cve* system. Table 1 lists the various genes tested.

The first step was to measure the interaction between a given gene and the *cve* complex. Crossvein defects were assessed in terms of the rating system already described, a rating from 0 (normal crossveins) to 12 (crossveins entirely absent). *cve* males were mated to females carrying a given gene, and the average rating of the progeny was compared to the average rating found in the F_1 of an Orgeon R × *cve* cross. The validity of this precedure depends on the similarity to the wild Oregon R genotype of the backgrounds of the stocks being tested. In extreme cases this was no problem, but intermediate results were questionable. Table 2 shows the results of these tests. It will be seen that the *cve* complex interacts strongly with a number of the genes tested.

The significance of the interaction is not easy to determine, however. In the first place, the *cve* complex interacts with *cv-c*. (This and succeeding symbols are explained in Table 1.) Does this mean that all *cve* flies are heterozygous for *cv-c*? Apparently not, for there is no 1:1 segregation for the easily recognizable *cv-c* phenotype (Table 1), nor do half the eggs die, as would be expected if the *cv-c* homozygotes were inviable with a *cve* background.

Moreover, previous crosses have shown that *cv-c* interacts with the *cve* complex in the absence of any third chromosome *cve* factor. Again (MILKMAN

TABLE 1

Genes known to affect posterior crossvein (p.c.) formation

Mutation	Locus symbol	Main effect	Other effects	Crossvein defects	
				Freq. percent	Degree
crossveinless	1–13.7 *cv*	anterior and posterior crossveins absent	.....	100	12
crossveinless-c	3–57.9 *cv-c*	p.c. defective: crossvein fragment parallels longitudinal veins	.....	100	10
crossveinless-d	3–65.0 *cv-d*	p.c. defective indistinguishable from *cve*	.....	100	10
detached	3–72.5 *det*	p.c. defective indistinguishable from *cve*	.....	†	0–8
Abruptex	1–3.0 *Ax*	longitudinal veins incomplete terminally	p.c. defective	*	0–6
dachsous	2–0.3 *ds*	wings smaller crossveins closer	p.c. defects body thicker	‡	0–?
Gull	2–12.0 *G*	wings held out and curved down	crossveins broken bristle duplications Vein L_1 thickened	*	0–?
dachs	2–31.0 *d*	wings smaller, crossveins closer tarsi four-jointed	p.c. defects body thicker	†	0–?
elbow	2–50.0 *el*	wings bent; alulae, halteres small; p.c. defective		100	6–12
divergent	3–20.0 *dv*	wings diverge	p.c. defects	†	0–4

* – variable.
† – rare unless selected for
‡ – apparently noted only in some alleles

1960c), *cv-c* does not increase the susceptibility to phenocopy production of crossvein defects. We are thus faced with the concept that mere positive interaction does not establish that the gene in question is part of the *cve* complex.

In addition, the reciprocal relationship applies. A negative result should not eliminate a locus from consideration. Interaction differences among the alleles at the *ds* locus illustrate this point (Table 2). Moreover, until we know how powerful each *cve* gene's effect is, judgment should be reserved since a weak gene's interaction could be masked by an unfavorable background.

This procedure of testing interaction is, however, of value. Genes that give a positive interaction may well be in the *cve* complex, and they can be subjected to further investigation. Five criteria can be applied: 1. Would the gene be included in selection for the phenotype? 2. Does the gene increase phenocopy susceptibility? 3. Does the expression of the gene's crossvein effect increase at lower temperatures? 4. Does the gene lie in a region shown to have *cve* activity by

TABLE 2

Ratings of female offspring of mutant females × cve *males*

Mutant or strain	Experiment	No.	Avg. rating
Oregon R (wild type)	1	816	0.36
	2	186	0.33
crossveinless-c		126	7.33
crossveinless	1	117	5.19
	2	97	3.52
ancestral wild strain		20	3.60
net black cinnabar	1	107	3.20
brown	2	51	3.18
divergent	1	48	3.02
	2	39	2.80
dachsous-wide	1	72	2.89
	2	63	2.86
crossveinless-d	1	60	3.18
	2	102	2.89
	3	71	2.16
white	1	94	3.33
	2	60	1.93
detached		22	2.41
curved		8	2.38
Abruptex		24	2.38
elbow	1	35	2.09
	2	45	1.96
	3	37	1.78
roughoid hairy thread	1	36	2.22
scarlet ebony claret	2	41	1.27
dachs black	1	39	2.26
	2	62	1.50
	3	48	1.27
dachsous^{38k}		65	0.98
cv-c allele	1	58	0.71
	2	105	0.64
	3	128	0.52
vestigial		20	0.45
rudimentary		77	0.42
Notch^{8}		31	0.40
veinlet	1	124	0.69
	2	61	0.25
	3	50	0.24
yellow cut	1	57	0.47
raspberry forked	2	41	0.30
ebony	.	80	0.36
clot	.	41	0.27
dachsous	.	14	0.14
Ratings of female offspring of cve *females* × *mutant males*			
Oregon R (wild type)	1	219	0.23
	2	99	0.23
detached	.	24	5.0
crossveinless-c	.	295	4.7
divergent	.	30	4.3
Mohler's crossveinless polygenic strain	.	34	3.0

In comparison with the Oregon R strain, some of the other strains seem to have genes which interact with the *cve* complex to produce crossvein defects. These interacting genes are not necessarily the mutants for which the strains are named. An average rating over 1.0 is taken as good evidence for such interaction.

crossover tests? 5. Is the gene's phenotype exactly like the *cve* phenotype, or is it, like that of *cv-c*, similar but distinct?

Several of the genes under consideration may be eliminated immediately by application of the first criterion. None of the genes which in homozygous state independently cause defects of the posterior crossvein—or any other clear-cut phenotype for that matter—could have been involved in the selection of the *cve* complex, for they would have been noticed. Thus *cv*, *cv-c*, *cv-d*, *el*, and *Ax* are ruled out but not necessarily possible alleles of these genes.

The results of the following experiment imply that no allele of *cv-c* is involved. The F_1 of a *cv-c* × *cve* cross was inbred in several cultures, propagated for a number of generations by random mass matings, and then selected for wild type. This method got rid of the *cv-c* and *fixed the* cv-c *allele present in the* cve *stock*. After several generations the stock was completely wild type, and it was carried on by mass mating for several months without further selection. It is probable that the *cve* genes were now back near the level at which they had been in the wild stock except for any one that might be close to the *cv-c* locus. When flies from this stock were mated back to *cve* males, no interaction was shown (Table 2, "*cv-c* allele"), so that this locus may be removed from further consideration.

On the other hand, various known mutant genes with less pronounced effects (such as *det*, *dv*, *d*, and *ds*) could easily fit into the picture. All have effects on the crossvein even though they are incompletely penetrant.

The clearest case is *det*, which resembles the *cve* complex genes in many ways. It has essentially the same phenotype; it responds to selection; and it has low fertility. Moreover, its expression and penetrance are greater at 18°C than at 25°C.

dv resembles wild type under some conditions. The various *ds* alleles are associated with a body and wing structure just like that of the *cve* stock. Therefore these genes could have been selected for without being recognized as *dv* or *ds*. Dachs, however, causes four-jointed tarsi, and this phenotype has not been observed in *d* × *cve* crosses. With the possibility that other alleles occur and that four-jointed tarsi may not be irrevocably tied to *d*, the locus should not be ignored.

These criteria merely tell us whether a certain gene could have been selected for in assembling the *cve* complex. To determine whether a gene is actually present in the *cve* complex, crossover tests must be tried.

Crossover tests: The use of *cv-c* as a "booster" simplified matters a great deal. Interaction with *cv-c* enables one to detect fewer *cve* genes than would otherwise have been possible (MILKMAN 1960b).

Two marked stocks were used: for the first chromosome, yellow (*y*, body color), cut (*ct*, wing form), raspberry (*ras*, eye color), forked (*f*, bristle form); and for the third chromosome, Roughened (*R*, eye texture), Dichaete[3] (D^3, wing form), bithorax-Dominant (bx^D, haltere form), Prickly (*Pr*, bristle form). All combinations for each chromosome are distinguishable.

One test of the X chromosome proved surprisingly simple. A single *cve* male was mated to a *y ct ras f* female. All the daughters had the same X chromosome

from the father, and they were mated to *cv-c* males. The experiment measured interaction, then, between factor(s) on one X chromosome and *cv-c*.

Although one might say that these factors are not necessarily identical with the *cve* factors, two facts argue in favor of the validity of this procedure. One is that the *cve* flies, in contrast to other stocks, contain factors on the three major chromosomes which interact with *cv-c*, and so interaction with *cv-c* seems to be one of the most general properties of the *cve* complex. The other is that the same result was obtained by MOHLER (1955) who looked for *cve*-type factors on the X of a similar stock without using *cv-c*.

Results, using the rating system previously described, are listed in Table 3. It may be seen readily that only one region is involved, the region very near or beyond the forked locus. Because of possible effects from other chromosomes even in the absence of the *cve* X, it is impossible to say definitely whether the region is near or beyond *f*. The best way of attacking this problem further would be to use markers in the region of *f*. One may disregard the possibility that *f* suppresses the *cve* effect. Were this so, one should expect some definite variation in the data for the various crossover types, due to the segregation of the hypothetical *cve* gene(s) at regions other than near *f*. That no such variation was encountered is taken as proof that the results may be taken at face value.

The third chromosome presents a more complex picture. At least two loci are involved, and the conclusions arrived at after the crossover tests are not to be taken as proven. The third chromosome dominant marker stock previously described, *R $D^s bx^p Pr$*, was used, with *cv-c* being used as a booster.

The rating system as described was used. Three methods for using the data in analysis were available: each crossover class might be graded merely on the percentage of crossveinless flies; the average rating; or the probit average rating which compensates for minus values.

TABLE 3

First chromosome crossover data

Crossover class	Total no.	No. *cve*	Percent	Avg. rating*
y ct ras f	264	31	12	0.52
+ + + +	418	216	52	3.18
y + + +	100	45	45	2.86
+ *ct ras f*	69	12	18	0.61
y ct + +	67	35	52	3.25
+ + *ras f*	103	17	17	0.40
y ct ras +	110	62	56	3.44
+ + + *f*	133	34	26	0.97
y + + *f*	16	2	12	0.81
+ *ct ras* +	22	9	41	2.82
All males	1,324	466	35	2.00
All females	1,591	651	41	1.81

* Only males showed crossover types. For method of determining average rating, see text.

Each method has its drawbacks. The percentage method does not utilize all available information. In both the percentage method and the average rating method all wild type flies are classified as zero, which results in inaccuracies when comparisons are made. The probit plot in this case is difficult to use because the distribution is not perfectly normal. Nevertheless, all three methods result in approximately the same answer, and it seems reasonable to assume that this picture is correct.

The probit method which was used to determine the particular localizations is described as follows. The offspring of 16 crosses were classed and rated, as shown in Table 4. These data were then put on a probit plot, and a "true" average rating for each class was estimated. Table 5 contains these data. In order to facilitate

TABLE 4

Third chromosome crossover data

Class	Total	Rating 0	1	2	3	4	5	6	7	8	9	10	11	12
RDBP	356	320	14	9	5	5	1	1	1	0	0	0	0	0
+ + + +	598	118	32	47	44	42	51	61	48	64	36	44	11	0
R + + +	203	83	16	24	18	16	18	11	7	3	2	3	2	0
+ *DBP*	263	188	19	14	12	13	4	5	0	4	3	1	0	0
RD + +	55	28	8	7	3	2	4	1	1	0	0	1	0	0
+ + *BP*	73	45	7	3	2	1	3	3	2	5	2	0	0	0
RDB +	182	138	21	12	4	2	2	2	0	0	0	1	0	0
+ + + *P*	290	110	17	19	18	21	22	30	17	15	10	5	5	1
R + *BP*	24	23	1	0	0	0	0	0	0	0	0	0	0	0
+ *D* + +	22	10	2	1	0	2	4	1	1	1	0	0	0	0
RD + *P*	12	10	0	2	0	0	0	0	0	0	0	0	0	0
+ + *B* +	16	5	2	1	3	1	1	1	0	2	0	0	0	0
R + + *P*	117	64	13	11	9	6	4	3	2	0	1	4	0	0
+ *DB* +	125	63	14	8	10	3	4	6	4	6	3	3	1	0
R + *B* +	2	1	0	1	0	0	0	0	0	0	0	0	0	0
+ *D* + *P*	8	6	0	1	1	0	0	0	0	0	0	0	0	0

Degree of crossvein defect is rated from zero (wild type) to 12 (posterior crossveins completely absent). Intermediate numbers represent intermediate degrees. *R*=Roughened (eyes). *D*=Dichaete (spread wings). *B* stands for bx^{D}= bithorax-dominant. *P*=Prickly (bristles). See text.

TABLE 5

Transformation of rating data to percentages of total third chromosome cve *effect*

Marker class	Avg. rating	Percent of difference between + + + + and *RDBP*	Marker class	Avg. rating	Percent of difference between + + + + and *RDBP*
RDBP	−4.3*	0	+ + *BP*	−0.2*	44
+ + + +	+5.1	100	*RDB* +	−2.0*	24
R + + +	+2.0	68	+ + + *P*	+3.0	78
+ *DBP*	−1.5*	30	*R* + + *P*	+0.6	51
RD + +	+0.7	53	+ *DB* +	+0.7	52

* Estimated from distribution. See text.

calculations, the ratings were converted to comparable values from zero to 100 as follows: the lowest rating (parental marked class) was −4.3, and the highest (parental unmarked class) was 5.1, making a range of 9.4. By setting −4.3 at zero and +5.1 at 100, it was possible to convert the other ratings by use of the formula:

$$\frac{\text{rating} - (-4.3)}{5.1 - (-4.3)} = \frac{\text{rating} + 4.3}{9.4}$$

The resulting fraction was multiplied by 100. Table 5 also lists the converted values for each crossover class.

Now the contribution of the *cve* chromosome region opposite each marker could be calculated merely by subtracting the value for a class with the marker from the value for a class identical except for not having that marker. Table 6 lists these calculations. It will be noted that four calculations may be made for each of the end regions, two for each of the inner ones. More are possible in each case, but these would involve values for very small multiple crossover classes and would be meaningless.

It should be noted that although no effort was made to adjust the data, values for reciprocal crossover classes equal close to 100 in each case. Moreover, the four values obtained for the four regions added up to about 97 before the figures were rounded off. This degree of internal consistency seems quite satisfactory.

In the analysis of these values, one assumption must be made; namely, there is no *cve* factor in region 2. As will be shown later, this region is only 8.6 units long,

TABLE 6

Calculation of the contribution of the region opposite each marker gene

For $+^{R}$:	$++++ - R+++ =$	100–68	$= 32$
	$+DBP - RDBP =$	30– 0	$= 30$
	$+DB+ - RDB+ =$	52–24	$= 28$
	$+++P - R++P =$	78–51	$= 27$
		$+^{R} =$	$\overline{30}$ (average)
For $+^{D}$:	$++BP - +DBP =$	44–30	$= 14$
	$R+++ - RD++ =$	68–53	$= 15$
		$+^{D} =$	$\overline{15}$
For $+^{bz}$:	$+++P - ++BP =$	78–44	$= 34$
	$RD++ - RDB+ =$	53–24	$= 29$
		$+^{bz} =$	$\overline{32}$
For $+^{P}$:	$++++ - +++P =$	100–78	$= 22$
	$RDB+ - RDBP =$	24– 0	$= 24$
	$+DB+ - +DBP =$	52–30	$= 22$
	$R+++ - R++P =$	68–51	$= 17$
		$+^{P} =$	$\overline{22}$

These values represent the percentage of the total *cve* effect of the third chromosome assigned to each region by comparison of effects associated with various chromosome marker combinations.

due to the suppression of crossing over near the *D* locus by an inversion associated with D^3. This assumption simplifies matters by implying that each marker locus is next to only one region with *cve* factors in it, and this enables us to localize the *cve* factors within their regions by comparing the contributions of the parts of the chromosome near each of the markers. The *R* and D^3 data serve for region 1, and the bx^D and *Pr* data for region 3. The ratio of contributions of the two marker areas is inverted to find the ratio of distances from each marker to the *cve* factor (if there is only one per region).

Region 3 is the simpler case. $+^{Pr}$ contributes 22 percent of the third chromosome *cve* effect, and $+^{bx}$ contributes 32 percent. The ratio of distances from a single *cve* gene (22/32) shows the distance to *Pr* to be greater. The distance from *bx* to *Pr* is 31.2. *Pr* is located at 90.0, so 32/54 of the *bx-Pr* distance would place the *cve* gene at 71.5. It is suggested that the gene in question is *det* (72.5), recalling the evidence previously presented. There are other possibilities; namely, two *cve* genes occur in region 3 with neither being *det*, or three, with one being *det*. These possibilities are predicated on the location of the center of the *cve* effect at 71.5. This effect contributes about 55 percent of the third chromosome's *cve* effect.

Region 1 presents difficulty. By the same method of calculation, a single gene could be at 12.1. There are no known genes between 1.4 and 19.2, however. The data are not sufficient to permit one to decide exactly where two genes would be, since their relative contributions would also be unknown. But one gene would clearly have to be very near *R*, between zero and 1.4. The other would lie between 19.2 and 50, the approximate place where crossing over is no longer suppressed by the inversion associated with D^3.

If there is only one gene on the left arm, it contributes 45 percent of the third chromosome's *cve* effect. If two genes are involved, one may at least calculate limits for their contributions. The gene not near *R* would have a minimum of 15 percent, the share assigned to $+^{D^3}$, if it did not recombine with D^3. If it were as far from D^3 as possible, at approximately 19, its contribution would be 25 percent, according to the method described above. The *cve* gene near *R* would contribute the rest, from 20 percent to 30 percent.

Since *dv* (at 20.0) is a logical possibility, and since no genes have been definitely localized in the region between 1.4 and 19.2, the number and location of *cve* factors on the left arm of the third chromosome are not as yet determinable. Further study with different recessive markers and using *Ax* (on chromosome 1) as the booster should bring out the answer.

Some investigators would consider the 1.4–19.2 region to be an area for polygenes. MATHER (1941) believes that polygenes are found in particular places, especially in blocks of heterochromatin (which this region, by the way, is not). GOLDSCHMIDT (1951) assigns a similar role to heterochromatin, particularly intercalary heterochromatin. DUBININ (1948) felt that the regions around net and blistered contain many polygenes. I cannot subscribe to the idea of special locations for polygenes for two reasons; one is that some polygenes have been

localized among "ordinary" genes; secondly, the intergradation between polygenes and other genes is quite smooth. There are very few genes in Drosophila with minor functions, as the lethality of almost all homozygous deficiencies shows (SINNOTT, DUNN and DOBZHANSKY 1958; GOLDSCHMIDT 1955); therefore, heterozygous deficiencies induced in this region should be expected to produce new recognizable phenotypes even if spontaneous mutations were generally so slight as to produce little morphological effect. We can clearly assume, then, that the *det* locus, near 72, is the site of the only *cve* gene in the right arm of chromosome 3. The left arm may contain one *cve* gene near 12 or two *cve* genes, one near locus one and the other between 19 and 50, possibly *dv*, at 20.

With respect to the second chromosome, data are available from matings made to test for crossover suppressors on this chromosome. For this purpose, net (*net*, wing veins) black (*b*, body color) cinnabar (*cn*, eye color) brown (*bw*, eye color) flies were used; only the non-*net* flies could be assessed for the *cve* phenotype. There is an indication from limited data that *cve* activity is present on both arms of the second chromosome.

The genes of the cve *complex:* We can say that there are at least five *cve* factors: one on the X, near *f* (56.7); one on the right arm of the third, *det*, about 72; one or two on the left arm of the third; one or more on the right arm of the second; and one or more on the left arm of the second.

It is further possible to state the relative contributions of the genes on the X and third chromosomes and of the second chromosome as a whole. From available data it is not possible to determine whether a *cve* factor involves a single gene or two or more closely linked genes. For the present, the simpler (one gene) interpretation is used.

The X chromosome, then, has one factor whose contribution can be estimated by comparing the true mean rating of classes with the X factor to that of classes without it. A probit plot of the data shows a difference of about 6 units which is the contribution, then, of the hemizygous X factor. Since the *cve* genes are semidominant by the criteria we use, a heterozygous X factor has a value of about 3 rating units.

The third chromosome has a value of 9 units in the heterozygous condition, with 45 percent of the value associated with the left arm and 55 percent with the right arm. Heterozygous *det* is then worth 5 units, and the left arm gene or genes worth 4. A rough estimate of the second chromosome's contribution, based on its approximate equivalence to the X in earlier crosses, would be 3 units per chromosome.

If the values for each chromosome are added together, the sum is 30. These 30 units cover the range between the $+9$ rating of the *cve* flies and the value -21. The latter is the value the wild stock would have if all *cve* genes were replaced by non-*cve* alleles in the laboratory stocks with which they were compared in the crossover tests. It is a measure of the reserve crossvein-making ability of the flies. The wild stock, then, could be selected to a norm of reaction measuring at least $9 - 30 = -21$ rating units. No basis exists here for distinguishing the maximum

crossvein-making abilities of the two sexes as was done with Oregon R (MILKMAN 1960b), because no *cve*-free line of the ancestral wild stock has been made.

Two indirect lines of evidence indicate that the base value of -21 should be approximately the same for both males and females. One is that in the early selection for crossvein defects, males and females appeared with equal frequency. Another is that, in contrast with Oregon R where females responded more than males, both sexes of the wild stock respond about the same to phenocopy treatment at 40.5°C to be described in a subsequent paper.

In summary, two further lines of attack on the characterization of the *cve* genes have been introduced. The interaction of the *cve* complex with various mutants has been used as a lead. Crossover analyses have led to conclusions of varying precision: that there is one *cve* factor on the X chromosome, near *f*; that there are two or three on the third, of which one is *det*, and that there are at least two on the second.

It has also been concluded that the *cve* complex is homozygous for these factors and that the wild ancestral stock's base value for crossvein making is about -21 for both sexes, as opposed to -13 and -19 for Oregon R females and males, respectively.

DISCUSSION

Using the *cve* complex as a model, we may now try to relate the gene to phenotype and to evolution. It is not the purpose of this discussion to set forth any new ideas, but rather to reaffirm those of the old ones which seem best to apply to the case at hand. The basis of the discussion will be the following set of facts: 1. Several genes have been found in a wild population which affect crossvein formation. 2. These genes act additively on a process underlying crossvein formation (MILKMAN 1960b). 3. This underlying process and a corresponding norm of reaction may be quantified. They vary over a wide range, depending upon the number of *cve* genes present (MILKMAN 1960b). 4. The part played by a single gene with respect to the norm of reaction is small. 5. The *cve* genes are not rare in wild populations. They are not to be regarded as less plentiful than their alleles, in general, and they are widespread (MILKMAN 1960a). 6. The number of genetic loci affecting crossvein formation is out of all proportion to the crossvein's morphological importance.

Considering first the relationship of gene to phenotype, the outstanding consideration is that many genes bear quite directly on the production of the *cve* phenotype. The *cve* genes are related to one another by their additive participation in defining the extent to which a developmental process is carried out, upon which crossvein formation depends. In this way, crossvein formation is like completely quantitative characters, which were once thought to have a monopoly on polygenes.

Because the *cve* genes do act additively, any of several combinations may achieve the same result, at least as relates to crossvein formation. Therefore, there are two ways in which the norm of reaction under discussion could be stabi-

lized in the population. One involves a fixed combination of genes in a homozygous state. The other involves maintaining a certain combination of heterozygous genes. The latter alternative apparently applies to the *cve* genes. The ready adaptability of this method would seem to reward its use, but it must be borne in mind that the quick morphological response to selection for crossvein defects would be of little value to the population if viability were markedly reduced, as was the case in these experiments.

The explanation of this heterozygosity would have to rest on single locus heterosis, since no single inversion is found in enough of the ancestral Amherst flies (IVES and HINTON 1946) to hold together groups of *cve* genes. Besides, the genes are on at least three chromosomes. The maintenance of such heterozygosity, which LERNER (1954) has strongly emphasized, entails a good deal of potential variability. This variability would seem to have two characteristics. Much of it is deposited in each member of the population and not in just a few, so that a small number of individuals picked at random would possess a major part of the population's variability. Also, the elements which could contribute to morphological change have already been tested physiologically. These elements are already a functioning part of the animal's genome.

The above considerations have led directly into the relationship of genes to evolution, for in this relationship the distribution of genetic variability is of extreme importance. The thinking on this subject has come far since the days when the material of evolution was thought to be sudden, rare mutations which were accepted or rejected by the environment. The great genetic variability within sexually reproducing populations has been discovered and the importance of recombination has been emphasized. Evolution is recognized as a population phenomenon, rather than a series of changes each in an individual. This trend in thought has made evolution seem much less a paradox; now the acceptance of a further step would add to our understanding of evolution. For it would seem that the genetic variability present in one individual is far greater than we imagined.

To the extent that the *cve* complex can be generalized, it can be said that each phenotypic variation among individuals in natural populations is controlled by many genes present in large numbers, rather than by single genes present in small numbers. Either alternative could account for the same amount of phenotypic variation in a population. The striking implication of the first alternative, however, is that potential variation among offspring of a few individuals would be on the order of that previously expected for the whole population.

When a hybrid corn plant is inbred a vast array of variation is seen in the offspring. The range of ear size, for example, is great because the parent was heterozygous for many loci affecting this phenotypic character. We know from many avenues of investigation that heterozygosity is great in Drosophila although little specific information has been obtained beyond the existence of this heterozygosity (DOBZHANSKY 1947, 1948; WALLACE *et al.* 1953; EPLING *et al.* 1953). It is difficult to see the value to an individual (upon whom selection works) of the various rare lethals and visible mutations floating in all populations. These cannot be

considered integrated into the gene pool, although their presence is due to the necessity and randomness of the process of mutation. The directly important heterozygosity and, therefore, the important potential variation must be high in frequency in the population.

The observed high frequency of the *cve* genes, the observed additive relationship of the *cve* genes to a process underlying crossvein information, and a survey of the nature of meaningful heterozygosity fit together to imply the following interpretation. A population does not store its variability in the form of rare genes that will spring into abundance when called upon. Rather, variation is brought about by the recombination of genes, many of which are present in any one individual, and most of which are present in any few individuals.

A striking feature of the Amherst wild population with respect to crossveins is this contrast. Although 99 9 percent of the flies have perfect crossveins, and the defects in the others are hardly recognizable, yet from the progeny of any 20 a strain can be quickly selected to give rise to a majority of *cve* members. Experimental tests strongly support this viewpoint (MILKMAN 1960a). DOBZHANSKY (1959) found that the variance of viability among the progeny of small samples of a population approaches the variation of the population itself. This combination of stability and adaptability is all the more striking when it is realized that the same situation probably holds for *Drosophila melanogaster* all over the world. Surely the potential variation is integrated into the genotype of the individual.

But just how general is this phenomenon? Does it apply only to crossveins and the few other recognized phenodeviants in Drosophila? It is too early to tell, but the means of inquiry are open.

The situation reminds one of the electric signs in which different combinations of lights can be lit to form an almost infinite array of pictures. No light is specific for any one picture; it may be used in countless combinations, each reducible to a numerical formula. The electric light has only two choices, on or off. At any genetic locus, the number of possible alleles is much greater. How reduced this number is in any one population remains to be seen, but clearly not too many could be present in significant frequencies. In practice, the choice might be between only two alleles, but perhaps groups of fairly equivalent alleles will be found to predominate (STERN 1943).

If a few individuals do possess as large a proportion of the population's potential variability as is here postulated, then this situation has remarkable experimental implications. For it means that most of the avenues of change immediately available to a population—and perhaps a large number of those immediately available to a species—can be found if the proper selection is applied to the offspring of a few individuals. Needless to say, this is no simple task.

It is clear that recombination of the existing genetic material cannot support evolution forever. It can only be suggested that whatever new genes are introduced to a population must have mild enough effects to be integrated into the gene pool—and individual genomes—in a fairly unobtrusive way before leading a charge to a new and striking phenotype.

SUMMARY

1. A true breeding stock, with polygenically-caused posterior crossvein defects has been selected from a wild strain of *Drosophila melanogaster*.

2. There are at least five additively acting genes involved: one near *f* on the X chromosome, *det* on the third chromosome, one or two on the left arm of the third chromosome, and at least one on each arm of the second chromosome. Localizations were done by crossover tests, using *cv-c* to enhance the expression of the *cve* genes. The relative contribution of each chromosome has been estimated.

3. The *cve* genes interact with many mutants to produce crossvein defects. The number of loci affecting crossvein formation is high.

4. The *cve* genes should not be thought of as rare. The widespread nature of genes affecting crossvein formation is discussed.

5. The natural importance of genes like the *cve* genes is discussed with particular attention to the type of genetic system where a number of heterozygous genes, each contributing a small part, govern a developmental process underlying the formation of a structure. The implications of such a system upon the distribution of potential variation within a population are described: any few individuals would have a large part of the significant variation present in the whole population.

6. The present susceptibility of natural genetic variation to detailed orthodox genetic analysis is suggested.

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NEW DATA ON THE GENETICS OF POLYMORPHISM IN THE SNAIL CEPAEA NEMORALIS L.

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Received September 9, 1959

A REVIEW of the remarkable polymorphism in the European land snail *Cepaea nemoralis* L. and of the various interpretations based on it has been given in a previous issue of this journal (CAIN and SHEPPARD 1954). Since the adaptive and evolutionary significance of the polymorphism is the subject of active research, a considerably better knowledge of its genetics is desirable. Subsequently, LAMOTTE (1954, 1959), and CAIN and SHEPPARD (1957) have provided more data. The purpose of this paper is to present further information from the breeding program being carried out at Oxford and to consider the significance of the dominance relations revealed.

Orange bands

This form is characterized by having both bands and lip, i.e. all parts normally with dark pigment, a brownish orange instead of dark brown or black. (This orange color is often found in the shells of snails with normally colored bands which have been badly scorched in grass fires, and must not be mistaken for the genetically orange form). The orange pigment may be missing in young individuals, so that they seem like hyalozonates (with unpigmented bands), but they develop the characteristic orange-brown pigment later in life. We have found this form only in one small locality in the Oxford district (in which it is not uncommon). One specimen which looks the same was seen in a sample from Bundoran, Western Ireland, very kindly shown to us by DR. A. COMFORT.

This form is recessive to normally-pigmented bands, since in mating ES 1 (Table 1) one normal-banded parent and one orange-banded gave only normally-banded progeny. This conclusion is confirmed by EX 1, in which no orange-bandeds were produced among 19 individuals, all of which are progeny of the orange-banded adult. The form is not phenotypic modification since it segregates from an orange-banded parent in EM 1. E 2 was set up with adults taken from the wild, but all eight progeny are orange-banded, and therefore must be the result of a mating between them.

All the orange-bandeds we have seen in the wild have been pale yellows, never pinks, although the pink allelomorph is common in this population. This suggests that there is linkage between shell color (pink and yellow) and color of banding, unless the gene producing the paler bands is also removing the pink or yellow pigment from the shell, producing pale yellows. Alternatively, the gene may not exert any effect in the presence of the allelomorph for pink.

TABLE 1

*General list of broods**

Brood no.	Parents	Progeny	
E 2	w 10345 ob Ad w 12345 ob Ad	Yw 12345 ob 8	
EM 1	w 00300 ob Ad	PP 00300 1 PP 12345 1 Yw 00300 ob 2 Yw 10345 ob 1	
ES 1	Yw 12345 ob Y 12345	Y 12345 15	
EX 1	w 12345 ob Ad PP 00300	PP 00300 2(2) PP 12345 3(1) Y 00300 6(1) Y 12345 3(1)	
L 1	Y 00000 DP 12345	DP 00000 8 DP 12345 11 PP 00000 4 PP 12345 9	1957 item 13 with additions.
L 2	DY 00000 DP 12345	DP 00000 9 DP 00300 6 DP 12345 5 PP 00000 11 PP 00300 3 PP 12345 4	1957 item 14 with additions.
L 4	B 00000 B 00000	B 00000 1 PP 00300 1	1957 item 15
L 5	Y 12345 PP 00300	PP 00300 1	
L 9	B 00000 } sibs, PP 00300 } prog. L 4	MB 00000 2 PP 00300 1	
L 10	PP 00300 } sibs, PP 12345 } prog. L 2	PP 00300 12 PP 12345 21 PY 00300 4 PY 12345 6	
L 11	DP 00300 } sibs, DP 12345 } prog. L 2	DP 12345 1 PY 00300 1 PY 12345 1	
L 12	DP 00000 } sibs, DP 00000 } prog. L 2	DP 00000 38 DP 00300 3 DP 12345 9 DY 00000 6	
L 13	PP 00000 } sibs, PP 00000 } prog. L 2	PP 00000 18 PP 12345 9 DY 00000 7	
LE 1	PP 00300 prog. L 5 Y 12345	PP 12345 3 Y 00300 1	
M 2	P 12345 PY 00300	PP 00300 22 PP 12345 10	1957 item 3.
M 3	P 12345 Y 00300	DP 12345 2 MP 00300 1 MP 12345 9 PP 00300 8 PP 12345 6 PP 02345 1 PP 00345 1	1957 item 4 rescored as far as possible, with additions.

M	5	PP	00000	PP	00000	1 } 1957 item 5.
		Y	12345	PP	12345	3 }
M	6	PP	00000	PP	00000	26 } 1957 item 6.
		Y	12345	PP	12345	15 }
M	7	P	00000	PP	00000	36 } Revision of extant
		Y	12345	PP	00300	13 } material: all were
				PP	12345	18 } MP in 1957 item 7.
M	8	B	00000	B	00000	2 } 1957 item 11:
		DP	12345 db	DP	12345	1 } pinks there shown
				PP	12345	2 } as MP.
M	14B	PP	00300 } sibs,	PP	00300	4
		PP	00300 } prog. M 2	PP	12345	2
				PY	00300	1
				PY	12345	2
M	15	B	00000 } sibs,	B	00000	2
		PP	12345 } prog. M 8	PP	12345	2
M	16	PP	12345 } sibs,	DP	12345	7
		DP	12345 } prog. M 8	DP	12345 db	1
				PP	12345	10
				PP	12345 db	4
M	17	PP	00300 } sibs,	PP	00300	7
		PP	00300 } prog. M 3	PP	12345	3
				Y	00300	2
M	18	PP	00000 } sibs,	PP	00000	12
		PP	00000 } prog. M 7	Y	00300	1
				Y	12345	1
ME	1	PP	00300 db prog M 3	PP	00300	10
		Y	12345	PP	12345	4
				Y	00300	10
				Y	12345	5
ME	2	PP	00300 prog. M 2	PP	00300	1
		PY	12345	PY	12345	5
ME	4	PP	00000 Ad? prog. M 7	PP	00000	11
		Y	12345	Y	12345	13
ML	1	DP	00000	DP	12345	20 } 1957 item 16 with additions:
		Y	12345	PP	00000	21 } MP all rescored as DP.
ML	2	MP	12345 probably DP but faded: prog. M 3	DP	00000	11
				DP	12345	5
				PP	12345	9
		PP	00000 prog. L 2	DY	00000	7
ML	3	DP	12345 prog. M 3	DP	12345	26 } See Table 2 for
		DP	12345 prog. L 2	DY	12345	11 } details.
MP	1	Y	00300 hz Ad	DP	00000	3 } Progeny of either
		DP	00000	DP	00300	1 } parent.
				Y	00300	17 Progeny of Y only.
MR	1	PP	00000	PP	00000	42 1957 item 8.
		Y	12345			
MR	2	Y	00000	PP	00000	15 }
		PP	12345	PP	12345	10 } 1957 item 9 with
				Y	00000	9 } additions.
				Y	12345	15 }
MR	3	DP	00000	DP	00000	45 } 1957 item 10.
		Y	12345	Y	00000	33 }

TABLE 1—Continued

*General list of broods**

Brood no.	Parents	Progeny	
MR 4	PP 0000¹0 B 00000	B 00000 9 B 000¹00 1 PP 00000 7 PP 0000¹0 1 Y 00300 2 Y 12345 3	1957 item 12: trace bands not then scored.
MR 23	PP 00000 prog. MR 1 Y 12345 prog. MR 2	PP 00000 41 Y 12345 48	
MR 28	PP 00000 } sibs, Y 00300 } prog. MR 4	PP 00000 3	
MR 30	DP 00000 prog. MR 3 Y 12345 prog. MR 4	DP 00000 36 Y 12345 22	
MR 31	DP 00000 prog. MR 3 Y 12345 prog. MR 4	DP 00000 16 Y 12345 19	
MR 32	B 000¹00 prog. MR 4 Y 00300 S prog. R 5	B 00300*S 1 B 00300 1 Y 00300 2 Y 00300*S 1 Y 02345 S 1	Very thin band. Too small for S.
MR 34	DP 00000 prog. MR 3 Y 00300 prog. R 5 Y lost: not scored for S	DP 00000 8 DP 00300 S 4 Y 00000 1 Y 00300 S 3 Y 00300 5	
MR 35	DP 00000 prog. MR 3 Y 00300 prog. R 8	DP 00000 9 DP 00300 2 Y 00300 11	
MR 36	PY 00000 Ad PP 00000 prog. M 6	DP 00000 16 PP 00000 7 Y 00300 6	
MR 38	PP 00000 prog. MR 1 DP 00000 prog. MR 3	DP 00000 10 PP 00000 7 Y 12345 1	
MR 39	PP 00000 prog. MR 1 DP 00000 prog. MR 3	DP 00000 13 PP 00000 4 Y 12345 6	
P 1	Y 00000 al Ad PP 00300	PP 00000 2 PP 00000 nl 1 Y 00000 1 Y 00000 al 2	Probably progeny of Y 00000
P 2	DP 00000 rl } sibs, DP 00000 } prog. PH 1	DP 00000 18 DP 00300 2 Y 00000 5	
PH 1	PP 00300 rl Ad	DP 00000 nl 1 DP 00000 rl 1 PP 12345 nl 1	

PLN	1	PP	00000 prog. P 1	PP	00300	1
		Y	00300 hz	PP	12345	1
				Y	00000	2
PM	1	PP	12345 prog. M 5	PP	00000	4
		Y	00000 al prog. P 1	PP	00300	4
				Y	00000	4
				Y	00300	2
POE	1	PP	00000	PP	12345	30
		Y	12345	Y	00000	28
R	1	B	00000	B	00000	18
		B	00000	DP	00000	2
R	4	B	00000	B	00000	7 } 1957 item 2, with
		B	00000	DP	00300	3 } additions.
R	5	B	00300 S at end	B	00300	18 Indistinct band.
		Y	00300	Y	00300*S	19
				Y	00300	1 Not scored for S (MR 34)
R	8	B	00300 Ad	B	00000	1
		B	00300 Ad	B	?	1
		P	12345	B	00300	2 } prog. B 00300.
				P	00300	1
				Y	00300	1
				B	00300	11
				P	00300	3 } prog. P 12345.
				Y	00300	7
RE	1	B	00300 prog. R 8 B 00300	MB	00300	11
				PB	00000	2
		Y	12345	PB	00300	1 Indistinct band.
				PB	00300	4 Full band.
				Y	00300*S	17
RE	3	MB	00300 prog. R 8	PB	12345	1
		Y	12345	PP	00300	4
				PP	12345	1
RL	2	MB	00000	B	00000	15 } 1957 item 1.
		Y	00300	PP	00300	24 }
T	2	MB	00000	DB	00000 nl	1
		PP	00300 rl, band hz or pale	PP	00300 al	1 pale band.
				PP	00300 hz?	1 small.

* Key:

Column 1: Brood number consists of letters giving provenance of stock followed by a serial number. E=Eynsham road, near Oxford; H=Hackpen, Wiltshire; L=My Lady's Seat, Wytham, Berkshire; LN=Lambourn, Berkshire; M=Marley Wood, Wytham, Berkshire; P=Pentridge, Dorset; PO=Portnoo, Donegal; R=Rockley, Wiltshire; S=Swindon road, Wiltshire; T=Thruxton, Hampshire; X=Uncertain origin.

Columns 2 and 3: B=Brown; P=Pink; Y=Yellow; (With prefix D (Dark), M (Medium), P (Pale). If the depth of color is uncertain, no prefix is given.); Yw=Very pale, whitened Y; w=White shell, color not ascertainable.

00000=Unbanded (0^1 means a trace of that band occurs near lip of shell); 00300=Mid-banded; 12345=Five-banded—i.e., with no band completely absent in the adult, unless otherwise specified. Fusions are ignored in this table.

al=Albolabiate; rl=Roseolabiate; db=Darkening bands; ob=Orange bands; hz=Hyalozonate; S=Spread bands. The absence of a symbol indicates that this character is normal. But with al or rl in column 2, the absence of a symbol or comment in column 3 means that the character is as yet unscorable in the progeny: where lip is known and normal, nl is used.

* Includes individuals which have parts of bands other than band 3 present, although carrying the gene controlling 00300.

Ad=Adult when mated; could have been fertilized already; Ad?=Adult; possible, but unlikely that it had been fertilized; prog. followed by brood number=parent is progeny from this brood.

Plain figures in column 3 are for progeny of the mating as stated; figures in parenthesis are of dubious parentage.

Hyalozonate bands ("albino" of several authors)

Of 18 banded offspring from the adult yellow hyalozonate in MP 1, not one was hyalozonate. This shows that hyalozonate is recessive to normal bands, as previously reported by LANG (1911).

Darkening bands

This form is characterized by the presence of bands which are not fully pigmented when they are first laid down, and consequently appear on the upper whorls of the shell as medium brown instead of deep brown or black. This dilution of pigment continues until the animal is approximately half-grown; thereafter, increasing amounts of dark pigment are laid down as successive additions to the shell are made, until on the last parts to be formed—next to the lip—full pigmentation is found. Even on the uppermost whorls, the bands are always much darker than in the orange-banded form.

Care must be taken in scoring this character when either the bands are very narrow, or there are varices present. In the first case, if the band is thin enough to be interrupted in places, it can be seen to be much paler close to the interruptions, where its width is least, than a little farther away; such very narrowed and often interrupted bands are what LAMOTTE (1951) has called "bandes pâles." He has shown them to be dominant to bands of normal width. Darkening bands are of normal width, or rather, the gene can affect bands irrespective of width, but its presence cannot be observed in bands which are so narrow as to be pale anyway. In the second case, it is well-known that if the animal stops making additions to the shell for a time, a line, or ridge, termed a varix, remains to show the limit of the shell before the pause, even after much more has been laid down. Such a pause often occurs when the animal is half-grown and hibernates in its first winter. When it starts in the spring, leaving a varix to mark the limit of the shell in that winter, the new part of the shell is often paler in ground color, and also in the pigmentation of the bands. The tone produced in the bands is often almost identical with that in a half-grown animal with darkening bands. As far as the bands visible on the last whorl are concerned, an adult animal with such a history looks like a very marked example of darkening bands; but just inside the mouth will be found a varix, and beyond that, the bands will be normally colored.

In M 8 a darkening-banded five-banded dark pink was mated to an unbanded brown, giving one dark pink five-banded and two pale pink five-banded, all with fully colored bands, (also two unbanded browns). One pale pink was sibmated to a dark pink (M 16), and gave the progeny shown, in which there is a clear 3:1 segregation for normal *vs.* darkening bands. Since dark pink and pale pink are allelomorphs (see below) and darkening bands appear in both dark pink and pale pink progeny, it cannot be controlled at the pink locus, and this brood gives evidence that the two loci responsible are not (or not very closely) linked. That darkening bands is recessive, as indicated by M 16, is confirmed by ME 1, in

which a normal-banded and a darkening-banded parent gave 29 offspring, all normal-banded.

Brown

That brown shell color is determined by an allelomorph at the locus controlling pink and yellow, and is dominant to these other colors (as already suggested by CAIN and SHEPPARD 1957) is supported by our additional breeding data.

Not only do broods L 4 (1957, item 15), R 1, and R 4 (1957, item 2) when summed give a 3:1 ratio, but whenever two heterozygous browns or one such brown and a yellow are mated (MR 32, R 5, RE 1, RE 3, RL 2 (1957, item 1)) only browns and one other color class segregate among the offspring. If brown were not an allelomorph at the locus controlling pink and yellow (nor extremely closely linked to it), both these colors as well as browns should appear in some broods, as they do in the brown × pink mating (MR 4). Furthermore, despite a slight epistatic effect of brown on banding (see below), it is clear from broods L 4, L 9, M 8, M 15, MR 4, R 4, and RL 2 that the locus determining the presence or absence of brown is very closely linked to that controlling banding, as is the locus controlling pink or yellow shell color (see below).

Spread bands and penetrance of banding in browns

The form 'spread band' has a wide middle band, often unusually heavily pigmented, with to either side of it, on the upper parts of the shell, traces of the other four bands, which may so extend and fuse farther down the shell as to give the appearance of a five-banded shell with complete fusion of all bands. In this case, the predominance of the middle band is still usually evident right up to the lip.

In R 5, the yellow parent had a strongly pigmented middle band, not unusually wide; the brown parent had a markedly widened middle band with a spread only at the mouth but with occasional traces of the other bands further back. The yellow offspring all have a very heavy middle band and all those scored have a definite spread on either side of it. The brown offspring have only an indistinct middle band, devoid of a spread and in some hardly visible. From these progeny were set up MR 32 and 34. In both, the spread-banded character has reappeared; in MR 34 it is segregating in the yellow class, and appears also in the pinks.

Since only one of the parents of R 5 had spread bands, and it reappears only in its yellow offspring, the character would appear to be dominant, with its allelomorph carried on the yellow chromosome of the brown parent.

In MR 32, the brown parent came from MR 4 which gave no spread bands in five yellow bandeds. The yellow parent came from R 5, had spread bands, and produced spread bands in the two scorable yellow progeny and in one brown. This suggests again that spread bands is dominant and exerts its effect in brown as well as yellow. The other of the two brown progeny is as yet too small for a certain score of banding, but is not as heavily banded as the first, and may even be not banded at all; this suggests segregation within the brown class, as might

be expected. The banded brown is apparently genetically abnormal in other respects (see below).

It might be objected that there is some interference between brown and the expression of the gene. The brown parent of R 5 supports this suggestion. If this is so, the spread-banded character is still dominant, but its linkage relationships remain undetermined. In RE 1, since the allelomorph is a dominant and appears in all the yellow progeny, one of the parents must be carrying it. The yellow parent is a normal five-banded; the brown has an exceptionally wide band but no traces of the other bands. The brown progeny have bands varying considerably from nearly normal width to very narrow, and correspondingly from fairly well-pigmented to very faint, and there are two which have no trace of any band. This appears to be a continuous variation. RE 1 also suggests from the appearance of the brown parent that the gene has sometimes a less marked effect in browns than in yellows. Since the brown progeny in both R 5 and RE 1 tend to have narrow or very narrow bands, not resembling the broad bands of the brown parents, it appears that they are not carrying the spread gene, which therefore is most likely to be linked with color, as suggested above. In RE 3, a mating between a brown narrow-banded 00300 from R 8 and a normal yellow five-banded, exactly as in RE 1, no spread bands appear in six progeny, and since no yellows appear, but brown and pink segregate, the brown could not have been carrying the spread gene, if as suggested the latter is linked to yellow. This mating, therefore, supports strongly the suggestion of linkage.

The suggestion that spread bands has less expression in browns is supported by the observation that normal middle bands is also reduced in browns. In RE 1, the two banded parents produced 35 offspring, of which two are phenotypically unbanded. From the known dominance of unbanded to banded, this is impossible; and in fact, these two seem to be only at one extreme of the continuous variation in the brown progeny, in many of which the band is markedly narrow, especially when compared with the yellows of the same brood. There is no doubt, therefore, that in this brood, the penetrance of midbanded in the brown class is not perfect. Similarly in R 5 the banding is weak in the brown class; in fact, when this brood was scored in November, 1958, five had no trace of a band. One developed a trace later, but this and the other four died when still rather small, and as they might or might not have developed bands they are not included in the table of broods. However, one of the RE 1 apparently unbanded browns is now over half-grown, and still has no trace of banding. The other is about one third grown. On the other hand, in R 8 (in the progeny of the pink 12345) the 11 browns all have well-marked bands, as expected. Similarly, in RE 3, the only brown offspring is banded as expected. The total number of browns expected to be banded is 50, of which two are unbanded.

Spread bands, therefore, appears to be a dominant, linked with color (in the matings reported, to yellow) and fully expressed in yellows. In browns, the expression of banding including spread bands, is sometimes reduced. Narrow band-

ing in browns may perhaps be a different allelomorph at the same locus, or only normal banding interfered with in browns.

The brown parent in MR 32 is unbanded with a very slight trace of band 3 almost at the lip. It was produced by MR 4, the brown parent in which was unbanded. However, it has given rise to two browns, one of which (the abnormal one mentioned above) is most remarkable in that the first few whorls are yellow 00300, and then change abruptly to brown with spread bands. Banded browns would not be expected at all in the progeny of this mating, unless there had been a crossover, or the brown parent is really banded genetically (as might be indicated by the trace of band 3). The change from yellow to another color in animals up to one quarter grown is shown by many yellow-spired individuals heterozygous for yellow, and this condition appears to be connected by continuous variation with ordinary yellow tip (see below). Until more offspring can be obtained from this mating, little more can be said about the penetrance of banding in browns.

Fusions and width of bands within the 12345 class

Mating ML 3 was between a :2345 dark pink and a 12345 dark pink. The progeny are shown with detailed scorings for banding in Table 2. The result

TABLE 2

Fusions and reductions of bands in brood ML 3

Parents: DP : $_{2345}$ × DP $_{1234_5}$			
Progeny:			
DP		DY	
12345	3	$_{123}45$	1
$_{1}2345$	3	$:_{23}45$	1
$_{12}345$	2	$:_{2}3_{45}$	1
$_{1}234_{5}$	2	$0^{t}:_{345}$	1
$_{12}34_{5}$	3	$0:34_{5}$	1
$:_{2}34_{5}$	1	$::_{3}(4_{5})$	1
$:_{2}3_{45}$	1	$_{123}(45)$	1
$0:_{2}4_{5}$	1	$:_{23}(4_{5})$	1
1(23)45	1	$(_{12})_{3}(45)$	1
1(23)(45)	1	$(:_{2}3)(4_{5})$	1
$_{1}(23)(45)$	1	$(:_{23}(4_{5}))$	1
$_{1}(_{2}3)(45)$	1		—
$(_{1}(23)(45))$	1		11
$:((_{2}3)(4_{5}))$	1		
$:(2345)$	1		
$:(_{2}3)(4_{5})$	1		
$(:234_{5})$	2		
	—		
	26		

Key: 12345 Full bands $1_{2}345$ Reduced band 2
1:345 Intermittent band 2 $10^{t}345$ Trace of band 2
123(45) Fusion of bands 4 and 5 (1(23)(45)) Fusion of 2 and 3, 4 and 5, with later fusion of all.
Fusions of bands are scored as at three-fourths grown; in adults this is therefore the condition at the level of the lip on the penultimate whorl.

demonstrates that both fusion of bands and the width of bands, and their reduction to almost absence in the case of bands 1 and 2, are multifactorially controlled, with no clear-cut segregation. The time of onset of fusions is also continuously variable. It is noticeable, however, that in none of the progeny (with two exceptions which are still very small) was any band clearly and wholly absent, a condition which, for some combinations at least (00300, 00345) is known to be controlled at a single locus.

Depth of pink

Previously (1957) we distinguished three shades of pink, dark, medium and pale. Although this classification is valid on color alone, the medium pinks include both individuals which are genetically dark, and some which are genetically pale. Our previous difficulties can now be cleared up.

The pink parents of both L 1 and L 2 (previously reported as items 13 and 14 (1957) now reported with additional progeny) judging from their offspring must be homozygous pink, and are dark. Both produced a segregation (1:1) of dark and pale pink. Consequently, either dark is dominant to pale pink, or pale is controlled by a modifier of pink at a different locus, brought in from the yellow parents. But in this latter case, either pale pink or dark pink must be heterozygous for the modifier in the progeny, but not both. However, the sib matings L 10, 11, 12 and 13 disprove the hypothesis of modifiers, since there is no segregation of dark and pale pink in broods with either both parents dark or both pale. Since all these parents were heterozygous for yellow, and the hypothesis of modifiers is disproved, dark and pale pink must be allelomorphs. The broods from L 12 and 13 demonstrate linkage between both shades of pink and banding. M 16 shows a backcross for pale and dark pink. On rescoring the previously reported offspring when they were more nearly full-grown, and by comparing them with the undoubted darks and pales of L 1 and L 2, we find that in the broods previously stated to have produced only pale pinks from a medium pink parent (M 5, M 6 and MR 1; items 5, 6 and 8 in our previous paper), the parents were in fact pale. There is no reason, therefore, to doubt the allelomorphism. In ML 1 (previously reported as item 16), the medium pink parent is in fact dark, and is producing a segregation for dark and pale, which confirms the allelomorphism since all are heterozygous for yellow; and the pale pink allelomorph is linked with unbanded, the dark pink with five-banded. The medium parent is dark also in MR 3 (reported as item 10). In M 8 (item 11) the progeny are one dark and two pale pinks.

In L 13, all the pale pink unbandeds are heterozygous for yellow, all the pale pink bandeds are homozygous. It is often observed that the presence of banding is correlated with a lightened ground color of the shell. Nevertheless, inspection of the homozygotes and the heterozygotes in L 13 shows that the heterozygotes are lighter in tone, and a more orange pink, while the homozygotes are darker and a more bluish, less orange pink. Presumably, if the homozygotes had been unbanded, the difference would have been greater. It is possible, therefore, that

some confusion might be caused by homozygous pale pinks looking as dark as a rather pale or somewhat faded dark pink. Dark pink heterozygotes seem to be dark; this explains the excellent segregations between dark and pale pinks in the progeny of L 1 and L 2.

It appears, then, that when whole broods are taken into consideration, there is no difficulty in scoring dark and pale pinks; but the scoring of unrelated individuals may not always be straightforward, and we find that living dark pinks may fade perceptibly over a period of a few years, so as to cause some confusion.

Color of tip in the pink and brown classes

In L 1 and L 2, all pink individuals in the progeny are heterozygous for yellow. The dark pinks all have a pink tip to the shell. By tip is meant the shell as possessed by the newly hatched young, plus about one successive whorl. The pale pinks mostly have yellow tips (and in fact the yellow may extend much further down the shell, but this is not a character in question here). In L 12 (dark pink × dark pink) we know that the pinks which are heterozygous for yellow are unbanded, those homozygous for pink are banded, because there is very close linkage between color and banding (see also below). All the progeny of this mating have pink tips. In L 13 (pale pink × pale pink), in which we have the same situation with regard to heterozygosity and banding, the nine homozygotes have pink tips, while 17 of the 18 heterozygotes have yellow tips, the remaining one having a pink tip. This strongly suggests that although heterozygotes cannot be recognized by tip-color in the dark pink class, in pale pinks the heterozygotes for yellow often have a yellow tip. This is amply confirmed by the rest of our data. The pink parents of M 5, M 6, and MR 1 have pink tips and are known to be homozygous pale pinks by their progeny. The pink parent of MR 2 is yellow-tipped and heterozygous. In M 16, in which the pale pinks are almost certainly homozygous, none out of 14 have yellow tips. For the pink progeny available in good condition for scoring and known to be heterozygous, the color of the tip is shown in Tables 3–5. Table 6 gives corresponding data for brown shells. Some tips are almost whitish, and cannot be described with certainty as pink or yellow. These are shown as pallid.

The breeding data agree well with the hypothesis that browns or pale pinks are heterozygous for yellow if they have a yellow tip. Table 3 shows that about two thirds of the pale pink/yellow heterozygotes can be recognized. The noticeable heterogeneity between broods suggests that different combinations of modifiers of dominance and/or different allelomorphs are found in the various stocks. Browns with a pink tip are heterozygous for pink. Pale pinks with a pink tip are usually, but not always, homozygous for pale pink. We find that our dark pinks seem to have a pink tip, whether heterozygous or homozygous, except in MR 36: in this brood, of 16 dark pink progeny, eight had a pallid and eight a yellow tip; the tips of the pale pinks were two pallid, five yellow. Both in pale pinks and in browns, there are a number which cannot be classified, the tips being pallid or brown. Apparently, dominance is often incomplete in browns (for both yellow

and pink) and usually in pale pinks (for yellow), but it is complete, as a rule, in dark pinks (for both pale pink and yellow). Brown heterozygotes (for pink or yellow) and pale pink heterozygotes (for yellow) can therefore be identified when wanted to set up matings.

These suggestions are confirmed by observation of random samples taken from

TABLE 3

Color of tip in pale pink/yellow heterozygotes

No.	Pink	Pallid	Yellow	Total
EX 1	..	3	5	8
L 1	..	..	13	13
L 2	..	5	4	9
L 5	..	1	..	1
L 13	1	..	17	18
LE 1	..	..	2	2
M 5	..	..	2	2
M 6	..	4	9	13
M 7	..	1	46	47
ME 1	3	4	7	14
ME 2	..	..	1	1
ME 4	..	..	11	11
ML 1	..	.	4	4
ML 2	1	5	3	9
MR 1	..	..	42	42
MR 2	7	..	..	7
MR 4	1	..	5	6
MR 23	..	11	20	31
MR 28	..	..	3	3
MR 36	..	2	5	7
MR 38	..	..	7	7
MR 39	..	..	4	4
PM 1	..	..	2	2
POE 1	14	12	..	26
RL 2	..	..	20	20
	27	48	232	307

In this table, and in Tables 4, 5 and 6, the brood totals do not always agree with the figure in Table 1: this is because Table 1 includes old records of dead progeny which are now faded and not scorable for tip color.

TABLE 4

Color of tip in pale pinks of broods known to have both homozygous pale pinks and pale pink/yellow heterozygotes

No.	Pink	Pallid	Yellow	Total
L 10	6	7	20	33
M 17	4	2	2	8
M 18	..	7	4	11
	10	16	26	52

All 2:1 segregations.

TABLE 5

Color of tip in pale pink homozygotes

No.	Pink	Pallid	Yellow	Total
L 13	9	..	..	9
M 16	9	5	..	14
	18	5	..	23

TABLE 6

Color of tip in browns of known constitution

No.	Brown	Pink	Pallid	Yellow	Heterozygous for:
MR 4 parent	..	..	..	1	yellow
MR 32 parent	..	..	..	1	yellow
MR 32 offspring	..	..	..	2	yellow
R 5 parent	..	..	.	1	yellow
R 5 offspring	..	..	3	15	yellow
R 8 offspring	..	5	..	4	pink and yellow (1:1)
RE 1 parent	..	..		1	yellow
RE 1 offspring	..	..	5	13	yellow
RE 3 parent	..	1	..	..	pink
RE 3 offspring	..	..	1	..	yellow
RL 2 offspring	..	..	..	15	yellow

the wild in which we have scored tip color in browns and those pinks that are undoubtedly pale. Since in the brown class, many known by calculation to be heterozygous do not show either pink or yellow, the observed ratio of pink and yellow tips must be compared with that expected from a calculation of the frequencies of the pink and yellow genes in that sample based on the observed phenotype ratios of pink, yellow and brown. The results are given in Table 7. It will be seen that there is excellent agreement between the observed and calculated ratios. This suggests that the penetrance of pink and yellow in browns is approximately equal, i.e., that an unscorable brown heterozygote, as far as penetrance is concerned, is equally likely to be heterozygous for pink or yellow.

Pale and dark yellow

Mating L 2 is of a deep yellow unbanded with a dark pink five-banded known from the progeny to be heterozygous for pale pink. Matings L 12 and L 13 from this progeny segregated out deep yellow unbandeds, in L 12 from dark pink unbanded parents and in L 13 from pale pink unbandeds. In L 10 and 11, pale yellow bandeds segregated out from pale pink banded and dark pink banded parents respectively. By far the most likely explanation is that dark and pale yellow are allelomorphs at the locus controlling the pink shades, and that the unbanded chromosome was carrying the allelomorph for dark yellow, the banded one that for pale yellow. Then from L 2, dark yellow is dominant to pale. If the

TABLE 7

Color of tip in random samples from the wild

Locality	Shell color	Tip color			Phenotypes of sample		
		Indeterminate (brown or uncertain)	Pink	Yellow	Brown	Pink	Yellow
Rockley East	Brown	42	19(19)	27(27)	88	83	42
	Pale pink	2 (pallid)	6	9			
Rockley 4	Brown	13	6(7)	20(19)	39	32	40
	Pale pink	2	7	9			
Rough Down	Brown	4	1?(0)	11(12)	16	1	28
Wytham Lane	Brown		2(2)	9(9)	11	16	26

The expected values are in parenthesis next to the observed value for brown shell with pink or yellow tips.

difference were due to a modifier of yellow unlinked with banding, broods ML 2 and M 14B, from L 2 and M 2 respectively, might well have disproved the hypothesis of allelomorphism, but neither did. In ML 2, the yellow offspring have the chromosome carrying unbanded from L 2; they must therefore all be dark yellow on the allelomorphism hypothesis, and the appearance of any pale yellows at all would have shown the existence of a modifier. The seven progeny were all dark yellow. In M 2, a pale yellow mated with a homozygous pink produced nothing but pinks, and two of these in mating M 14B produced pinks and three yellows, all pale. Again, the occurrence of a single dark yellow would have disproved allelomorphism. Further support is given by ME 2, in which a pink from M 2, heterozygous for pale yellow on this hypothesis, was mated to a pale yellow, and segregated pinks and pale yellows (five), as would be expected.

The pale yellows in these matings have quite dark yellow tips; it is not possible, therefore, to separate dark yellows heterozygous for pale yellow by tip color.

Viability

Before crossover values can be calculated, the data must be examined for evidence of differences in viability disturbing segregation ratios. Moreover, since a stable polymorphism is in question, which is maintained despite visual selection in many localities for monomorphism, CAIN and SHEPPARD (1954) have pointed out that there is almost certainly strong heterosis acting to preserve it. Such physiological selection may be operating even under a careful breeding regime (although not necessarily in exactly the same direction as in the wild).

The data (Table 1) are unfortunately not sufficient to show small viability

effects which would be very important in the wild. Our findings can be summarized as follows:

Color:	
Pink/yellow heterozygote	Backcrosses to yellow, no significant departure from 1:1, nor heterogeneity between families. F_2, a nonsignificant deficiency of the recessives (yellows), no marked heterogeneity between families.
Brown/not brown heterozygote	Backcrosses, no significant deficiencies, nor heterogeneity. F_2, no significant deficiencies, a possibility of heterogeneity (R 1) but the numbers are small.
Banding in pink and yellow:	
All banding/unbanded heterozygote	Backcrosses, no significant deficiencies, nor heterogeneity. F_2, there is a not quite significant deficiency of the recessives (bandeds) but as this locus is linked with color, the deficiency could be due in part to that of yellows noted above.
00300/banded heterozygote	Backcrosses, no significant departures from an over-all 1:1 ratio, but considerable heterogeneity between families ($P<0.01$). F_2, not sufficient data.

(No test of banding in browns has been made since the degree of penetrance is in doubt.)

The heterogeneity between families in 00300/banding segregation is remarkable, the more so since there is no significant heterogeneity when all types of banding taken together are compared with unbanding, although this includes several families segregating unbanded and 00300. The heterogeneity could be due to linked semilethals, sometimes in coupling and sometimes in repulsion, or the allelomorph controlling 00300 may be particularly sensitive in viability to differences of conditions in the different breeding pots. It cannot be due to differences in penetrance, since at least part of it is caused by an excess of 00300, and part by a deficiency, whereas with incomplete penetrance a departure in one direction only would be expected. The occurrence of phenocopies is highly unlikely since the phenotypes are clear-cut, and 00300 never occurs in families in which both parents are known by inspection of their parents and siblings not to be carrying the gene for 00300.

Independence of 00300 and color

Broods EX 1, LE 1, ME 1 and ME 2 give no evidence of linkage between color and the gene for 00300. ME 1 and 2 are of particular importance, since if there were linkage, the allelomorph for 00300 must be on the chromosome carrying the allelomorph for yellow. The results of the two broods can therefore be

summed, and give an excess of the putative crossover classes pink 00300 and yellow 12345. This confirms our previous findings and those of LAMOTTE (1954) that 00300 and color are not, or are only loosely, linked.

Crossing over between color and banding

The following matings show that crossing over is extremely infrequent: ME 4, MR 23, 30, 31, 34, 35 (backcross coupling); PLN 1, POE 1 (backcross repulsion); M 18, MR 38, 39 (F_2 coupling); L 12, 13, P 2 (F_2 repulsion); ML 2 (single backcross repulsion). In fact, there is no evidence for it at all, except in MR 34 and 35. In both of these matings, the parent in which crossing over occurred came from MR 3. Because of the suggestion of a disturbed segregation ratio in the F_2, it is convenient to calculate crossover values from backcrosses only. The crossover value calculated from the backcross data is about 2.25 percent. Since no crossovers appeared in the F_2 families, the real crossover value is probably lower than this. FISHER and DIVER (1934) have reported differences in crossover value between families, but, as LAMOTTE (1954) has pointed out, they were using adult snails which might have been previously fertilized. Since all our crossovers occurred in only two matings in which the heterozygous parents were sibs, there is almost certainly heterogeneity in our data, and large differences in crossover value between different individuals.

Dominance relationships

The results so far obtained of genetical work on this snail have revealed a series of allelomorphs at the color locus, with a dominance hierarchy, in the order: brown, dark pink, pale pink, dark yellow, light yellow. In this, every form is dominant to all those to the right of it (except for tip color). It is remarkable that the series is also in order of decreasing darkness from left to right. If, as seems possible, orange bands is dominant to hyalozonate, there will be a similar series for the color of the band pigment, in the order full pigment (nearly black), orange bands, unpigmented bands (hyalozonate). These seem to be linked with the color locus. The modifications 00300 and 00345 of five-banded, however, are dominant, and unlinked with color, and LAMOTTE's "bandes pâles" is also a dominant to normal banding and unlinked with color. Since 12345 is linked very closely with color, it is perhaps not surprising that modifications of the width of the bands, and modifications removing some of the bands, are not linked, since they can appear only in the five-banded form 12345, and therefore cannot help but behave in part phenotypically as though linked with color. Thus in a locality in which pink unbanded was at an advantage to pink 00300 but yellow 00300 was at an advantage to yellow 00000, the population could become almost completely composed of P 00000 and Y 00300 by selection for the chromosomes P 00000 and Y banded, despite the fact that 00300 is not linked to color. Selection to increase the linkage between 00300 and color, therefore, would have little effect. Linkage of banding with color, however, is to be expected, since different combinations of bandings and colors have very different selective values against different backgrounds (CAIN and SHEPPARD 1954).

The dominance relationships in the series of allelomorphs at the color locus need explanation. It is not sufficient to say that a more intense pigment (e.g. deep pink, or the violet that is found in the calcareous layers of the shell in browns) will overpower a lighter or less intense pigment (e.g. pale pink or yellow) and that therefore the hierarchy is what would be expected if there were no dominance. One would expect the heterozygote between dark pink and dark yellow to be a *very* yellowish orange, even supposing the heterozygote to produce as much pink pigment as the homozygous dark pink, but the two are indistinguishable. Moreover, there is nothing in darkness or intensity of pigment as such that must be correlated with dominance; both dominant and recessive melanics are well-known in various sorts of animals. Nor can the difference between brown, pink and yellow be a simple dosage effect since the different colors must be produced by different pigments and not by a dilution of the same pigment (SHEPPARD 1958, p. 142).

In cases of polymorphism maintained by mimetic resemblance, a new allelomorph or gene affecting the visible appearance of the mimetic forms will not spread by visual selection unless either (1) it improves the present mimicry or (2) it produces a new phenotype which has a sufficient, although no doubt rudimentary, resemblance to a model not thus far exploited by this species of mimic. The visible influence on the new form of the genes mediating the already existing polymorphism will be eliminated in order to improve the new mimic, and thereby dominance will be evolved. Consequently, there will be a dominance hierarchy in mimetic polymorphisms in respect of all those allelomorphs that coexist in the same population. This has been demonstrated in detail by CLARKE and SHEPPARD (1960) in *Papilio dardanus*.

It seems not impossible that the polymorphism in *Cepaea nemoralis* can be regarded as a cryptic polymorphism analogous to the mimetic polymorphism in *Papilio dardanus*. It has been shown (CAIN and SHEPPARD 1950, 1952, 1954; SHEPPARD 1951, 1952) that yellow shells are at an advantage in green situations, pink ones in leaf litter, and brown ones in beechwoods with their frequent expanses of blackish soil. It is certainly true, at least in Britain, that plants at ground level are either green and growing or brown and decaying. Intermediates are not common, or common only for a short time in the year. If yellow shells are imitating the green elements in the background, and pink and brown ones the brown elements, it is understandable that intermediates between the yellows and the other colors which would be bright or dull orange, might nearly always be at a disadvantage. This sort of explanation might also apply to the difference between the pinks and browns, since dead vegetable matter is certainly often either straw-colored or blackish brown, but this point needs further investigation. It is therefore likely that there are fewer optimal phenotypes in Cepaea than there are possible combinations of the allelomorphs necessary to produce these phenotypes. When there is more than one optimal phenotype, polymorphism may evolve by disruptive selection (MATHER 1955). We can generalize the argument just given, and say that when the number of optimal

phenotypes is less than the number of genotypes necessary to produce them, a dominance hierarchy will also be evolved, with the ancestral form very low in the hierarchy.

It is quite possible that the bright sunshine and comparative aridity of much of France, especially in the south, by producing mainly pale backgrounds, favors yellows above the other colors, and that dark browns are confined to northern Europe because sodden blackish elements are far more frequent in the backgrounds there, although other causes may also be active. The hierarchy would also suggest that, irrespective of their present distributions, the allelomorphs dominant to yellow are later than it, brown being the latest of all, and that the original habitat of Cepaea was green places, perhaps grassy limestone pavements, in a warm climate, the animals probably aestivating in the height of the summer when the vegetation might be parched.

SUMMARY

1. The following genes are described:
(a) Orange banded, recessive to normal pigmentation of bands, probably linked with shell color.
(b) Darkening bands, recessive to normal pigmentation of bands, not closely linked with shell color.
(c) Spread bands, dominant in the 00300 phenotype, almost certainly linked with shell color.
(d) Dark versus pale yellow, allelomorphs at the pink/yellow locus, with pale yellow recessive.
2. The recessiveness of hyalozonate to normal bands is confirmed and the independence of 00300 and shell color is strongly supported. Pale pink (recessive) is allelomorphic to dark pink. Further evidence is presented that brown is allelomorphic with and dominant to pink and yellow.
3. Fusions and reductions (but not absences) of bands in the 12345 phenotype are multifactorially controlled.
4. There is an epistatic effect of brown on banding, reducing the expression of banding, so that it is even absent in a few cases.
5. Dominance is incomplete in many brown/pink and brown/yellow heterozygotes, and in most pale pink/yellow heterozygotes, but appears nearly complete in dark pink/yellow heterozygotes.
6. A striking heterogeneity between families in 00300/12345 segregation ratios is reported.
7. Data confirm that pink and banded are very closely linked. A few crossovers have been observed, but only in the progeny of two full sibs, which suggests heterogeneity in crossover value.
8. The significance of the dominance relationships at the shell color locus in this polymorphic snail is discussed. It is suggested that in polymorphisms whenever the number of optimal phenotypes is less than the minimum number of combinations of the major genes necessary to produce them, dominance will be evolved.

ACKNOWLEDGMENTS

We are grateful to DR. E. B. FORD, F.R.S. who read this paper in manuscript and to PROFESSOR K. MATHER, F.R.S. who read it and gave valuable advice on calculations for heterogeneity in crossover values. We also wish to thank the Nuffield Foundation for extensive support.

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PLEIOTROPIC EFFECTS OF A MUTANT AT THE P LOCUS FROM X-IRRADIATED MICE[1]

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Received September 14, 1959

BY means of the well-known method of irradiation followed by testing with recessive-marker stocks, many visible mutants at specific loci have been obtained in mice by several investigators. In some instances the mutants so obtained are presumably identical with the markers employed in the tester stock, but very often distinctive new alleles are involved. These latter may be of intrinsic interest in regard to knowledge of the locus, and they may also be useful additions to the available supply of markers.

Numerous mutations at the *p* locus ("pink-eyed-dilute") in linkage group I of the mouse are on record (GRÜNEBERG 1952; CARTER 1957; RUSSELL and RUSSELL 1959). Most of these resulted from irradiation of spermatogonial cells. RUSSELL and RUSSELL (1959) state that 14 of 22 such induced mutants in their studies were carried to homozygous condition, but no detailed characterization of any has been reported. In the present study such a characterization has been completed. The unusual degree of pleiotropy revealed makes the mutant significant and probably valuable for further studies, particularly of spermiogenesis and odontogenesis.

MATERIALS AND METHODS

The highly inbred albino strains "S" and "Z" were selected for irradiation. Aside from the albino gene, *c*, they possess no other mutant markers in their genotypes. Tests for induced recessive mutants were made by crossing irradiated individuals with mice of the "P" stock, which is homozygous for the marker genes *p*, *d* (dilute), *se* (short ear), *a* (non-agouti), *b* (brown) (CARTER *et al.* 1952) and also *m* (misty) (HOLLANDER, unpublished).

Since it was desired to induce mutation in mature sperms for this study, adult males were introduced into individual plastic tubes. These tubes were of one-inch inside diameter, with perforations for ventilation, and stoppers to prevent movement. To shield the anterior two thirds of the body, a lead plate was placed over the tubes. The dosage of X-rays employed was 500r from a G. E. Maxitron, 250 pkv, 30 ma, 0.25 mm Cu and 1 mm Al filters, at about 170r/minute. This dosage is sufficient to induce dominant lethal effects in nearly half the functional sperms (HOLLANDER and GOWEN 1955).

[1] Journal Paper No. J-3722 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa Project No. 1187. This work has received assistance from Contract No. AT(11-1) 107 from the Atomic Energy Commission.

Immediately after X-raying, each male was placed in a breeding box with four tester females. Since the male generally became sterile within about 15 days, it was discarded at about this time.

If no mutation were induced, the F_1 would be all wild type. A mutation induced at the *p* locus in a sperm would produce a phenotype resembling in some degree that produced by homozygous *p*, but presumably no other loci would be involved. Since the *p* and *c* loci are both in linkage group I, the constitution of such an F_1 individual for these loci should be $p^* \; c/p+$, where p^* represents the induced mutant. Chromosome aberrations (deficiency, translocation, etc.) are assumed not to be involved unless demanded by other evidence.

In order to study the phenotypic effects of the induced mutant when homozygous, one must first go through several generations of outcrossing and testing. Since the *p* and *c* loci are about 14 units apart, we assume that most of the progeny will be noncrossovers, and those receiving *c* will also get p^*. To detect which of the outcross progeny received *c*, the original F_1 showing mutation at the *p* locus was crossed with a homozygous chinchilla ($c^{ch} \; c^{ch}$) male. The c^{ch}/c progeny were light chinchilla in color, in contrast to the wild type $c^{ch}/+$. Further outcrossing of two of the presumed $p^* \; c/+ \; c^{ch}$ individuals to $C^+ \; C^+$ propagated the stock; inbreeding of the descendants produced $p^* \; c/p^* \; c$ and $p^* \; +/p^* \; c$ individuals for further investigation and breeding. The unexpected tests necessary in that connection will be detailed later in connection with treatment of the pleiotropic effects.

The mice in this laboratory are housed in an air-conditioned room; the boxes are of aluminum, with a litter of fine wood shavings, changed once a week. Food hoppers have a 1/4-inch mesh galvanized hardware-cloth side through which the mice gnaw the food biscuits (Purina Lab Chow and Wayne Lab Blox). Young mice are ordinarily weaned at the age of about four weeks. Other details of care and conditions have been typical of most mouse-breeding laboratories.

OBSERVATIONS

The F_1 mouse showing mutant pink-eyed phenotype was a vigorous female with normal fertility. She was the only such mutant observed in the F_1 population of about 3500 individuals, and there were 14 additional normal progeny from her sire, an S strain male. The litter in which she appeared was born on March 9, 1955, 23 days after the sire's irradiation.

The breeding program outlined above was followed without difficulty. Crossing over values between p^* and *c* were approximately 15 percent, in very good agreement with the map distance for these loci. Therefore no chromosomal disturbance of any consequence can be postulated.

In the outcross program, this female's descendants were ordinarily of large size and high prolificacy. By contrast, the $p^* \; p^*$ segregates proved small and sterile *inter se*. Accumulated records provide the following comparisons:

(1) Growth and body weight: Normals of this family ranged to over 50 grams in mature body weight, achieved within four months. Many were over 40 grams.

None of the p^s p^s mice ever reached 40 grams; one exceptionally fat male weighed 38 grams, while the majority did not reach 30. Growth curves showed that the weight differential was present at birth and was consistent to maturity, and amounted to about 30 percent less than the normal litter mates.

(2) Behavior: The p^s p^s mice were active but somewhat uncoördinated in movements. In some cases the activity was almost choreic (waltzing).

(3) Chewing: After weaning, some p^s p^s mice starved before it was realized that they were having extreme difficulty in chewing the food biscuits through the 1/4-inch mesh of the hopper. Provision of a 1/2-inch gap obviated this difficulty.

(4) Reproduction in females: Although p^s $p^s \times p^s$ p^s matings produced no young, crosses of p^s p^s females with normal males were more successful. At least half of the females however failed to breed, and the others produced only one to four litters. The maximum litter size was 11 young, while larger numbers were often obtained from normal sisters. The p^s p^s females rarely kept the young in a nest, but usually scattered and did not nurse them. Although several litters were raised, the young were very poorly grown. It was found that fostering young of p^s p^s mothers with normal nursing females was the best policy.

(5) Reproduction in males: As noted earlier, matings of p^s $p^s \times p^s$ p^s produced no progeny and apparently no pregnancies. Individual tests of the p^s p^s males with normal females also indicated sterility. For a more thorough test, over 80 p^s p^s males were mated with normal females, usually four females per male, and left at least a month. Only two males sired litters: in one case two litters, of four and one young, respectively, and in the other case a single young, dead at birth. The two males were left for a considerable time with additional mates, but appeared sterile.

Examination of the semen of p^s p^s males revealed another excellent reason for sterility—high proportions of the sperms were very abnormal in head structure. Details of sperm morphology are to be reported in a separate paper. The abnormalities varied from slight shortening of the hook and posterior end of the head to complete disorganization with filamentous protrusions. The abnormalities were found even in the testis, and have been traced to faulty acrosome development in the spermatid.

In still another test, 12 p^s p^s males were mated with four normal females each, and daily examinations for vaginal copulation plugs were made for two weeks. No plugs were discovered. Anatomical study of these males revealed no definite abnormality of the reproductive organs.

(6) Teeth and aging: As the p^s p^s mice reached what in normal mice would be the "prime of life," about the age of five months, signs of senility had appeared. Many, especially females, became emaciated and died, apparently as a result of difficulty in chewing. This difficulty was not a matter of inability to get at the food, but improper wear of the incisors, sometimes with obvious malocclusion. Instead of the usual bevelling wear, the upper incisors generally developed a transverse groove into which the lower incisors pressed. In some cases an

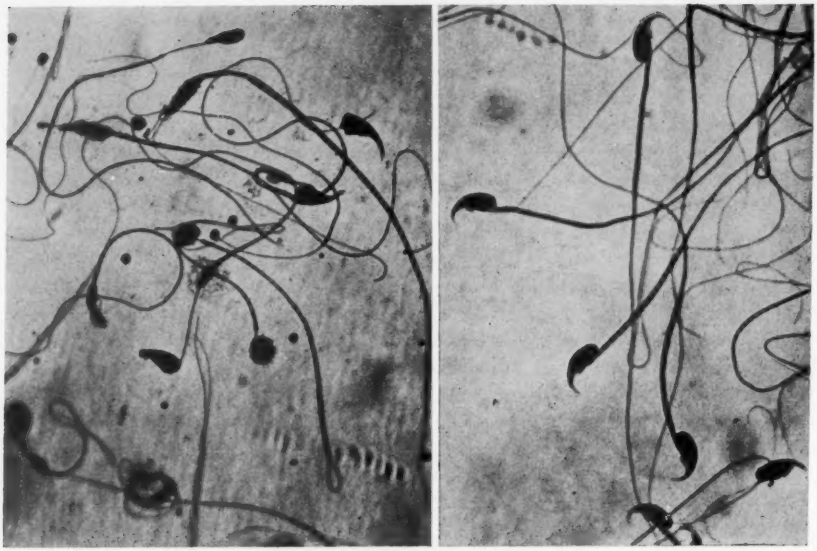

FIGURE 1.—Comparison of sperm head morphology of sperms from a p^s p^s male (left) with those from a normal male (right). Note that the normal hook and rounded posterior end of the head are altered or obliterated in the abnormals, often leaving only a loose mass of threads.

incisor broke off. Most of the mice were in such poor condition by the age of ten months that they died or were killed. Hardly any have lived over a year; one lived to 16 months. By contrast, normal mice of this stock readily live to a year and a half. A mash diet, which might have been more favorable for the p^* p^* mice, has not been tried.

DISCUSSION

The mutation under discussion, which will now be symbolized p^s, must be considered detrimental though it cannot be classed as a lethal. It was noted that heterozygotes ($+/p^s$) in this stock were very vigorous and prolific, but this fact seems more readily attributable to the strain crossing involved in propagating the mutant than to overdominance at this locus. There is no evidence indicating that chromosome damage was involved, because linkage of *p* and *c* loci was undisturbed, giving the usual crossover values.

In RUSSELL and RUSSELL's report (1959), of the 14 radiation-induced mutants at the *p* locus which they tested homozygous, six were lethal before weaning age. These were all from irradiated spermatogonia. Other evidence, particularly the lethal mutants obtained at the *d* locus on chromosome II, without involving the *se* locus extremely close to it, led the RUSSELL's to conclude that deficiencies are rare in such material, and that point-mutant lethals are realities. RUSSELL,

FIGURE 2.—Wear patterns of incisor teeth. A.—Normal, aged nine months. B, C, D.—Examples of wear patterns of p^s p^s mice at the same age as A, or younger. These abnormal wear patterns are associated with difficulty in chewing.

BANGHAM, and GOWER (1958) found a higher incidence of induced deficiencies in irradiated postspermatogonial stages. It is commonly assumed that deficiencies must be lethal when homozygous, since this has been the general experience with Drosophila.

If we assume that p^s is not due to a deficiency, the multiplicity of its effects in the homozygote constitute an apparent case of "genuine" pleiotropy. These seem impossible to fit into the one-gene-one-enzyme hypothesis of gene action, and similar theories, namely that all the effects can be traced to a single derangement of a primordial physiological process. It is at present impossible to conceive of such a common source of the changes here observed in pigmentation, growth, behavior, tooth wear, and spermiogenesis. The ordinary p allele, so far as has been discoverable, affects only black melanin pigment formation.

The relation of pseudoallelism to the question of pleiotropy has been well-discussed by GOLDSCHMIDT (1955, pages 398–400). There is no possibility of properly testing this aspect of the question in the mouse at present.

SUMMARY

A presumably X-ray-induced mutation at the p locus, recessive to both p and wild type alleles, has been analyzed. When homozygous the mutant produces the same effect on pigmentation as $p\ p$, but in addition there is poor growth, slightly uncoördinated behavior, difficulty in chewing, inadequate maternal care by females, sterility in males, premature senility, and abnormal wearing of the incisor teeth. The male sterility is not quite absolute, and is partly the result of poor mating response and partly a very high proportion of sperms with abnormal head structure. Since chromosome aberration seems not to be involved in the origin of this mutant, it is considered a new allele with unusual pleiotropic effects, and is symbolized p^s.

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CYTOGENETIC ANALYSIS OF THE ACTION OF CARCINOGENS AND TUMOR INHIBITORS IN DROSOPHILA MELANOGASTER. VII. DIFFERENTIAL INDUCTION OF VISIBLE TO LETHAL MUTATIONS BY RELATED NITROGEN MUSTARDS

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Received September 22, 1959

THE analysis of the mutagenic properties of various alkylating compounds on the testis of adult Drosophila, has revealed some differences in their mode of action (FAHMY and FAHMY 1956). The over-all mutagenic potency was shown to depend not only on the chemical reactivity of the alkylating groups, but also on the molecular configuration of the nonalkylating (or prosthetic) moiety of the molecule. The significance of the prosthetic moiety was also manifest in the relative mutagenic response of the various cell stages of the germ line. Thus among closely related 'nitrogen mustards' the amino acid derivatives were found to be mutagenic on all stages of the male germ line, including the spermatocytes and spermatogonia, while the corresponding carboxylic acid derivatives were practically ineffective on the younger cell stages, especially the gonia (FAHMY and FAHMY 1958b, 1959b). This difference was attributed to an intracellular 'transport' mechanism connected with protein synthesis, and favoring the conveyance of mutagens with an amino acid moiety to sites of nucleoprotein construction.

The above differences were based on the analysis of one class of mutations (the recessive lethals) and may therefore be looked upon as mainly quantitative—due to various degrees of attack on the same genic sites. However, it has now been demonstrated (FAHMY and FAHMY 1956, 1959a) that mutagens belonging to different chemical series do exert differential action on various genes, especially as regards the yield of visibles. It was, therefore, of interest to determine whether differential mutagenicity could also occur between compounds within the same chemical series, i.e., among the mustard derivatives of closely related compounds. An observation favoring this possibility is the higher ratio of visibles to lethals induced by the mustard derivatives of amino acids compared to those of related carboxylic acids (FAHMY and FAHMY 1956). Most outstanding in this connection is that the mustard derivative of phenylalanine [p–NN–di–(2-chloroethyl) aminophenylalanine] was found to induce a ratio of 3 visibles:10 lethals, which

[1] Acknowledgment is given to PROFESSOR A. HADDOW for permission to have the printing costs of extra pages of this communication charged to the Institute of Cancer Research, Royal Cancer Hospital.

is significantly higher than the corresponding ratio for any other alkylating agent as well as radiation. In view of the great theoretical and practical implications of this discovery, the phenomenon was subjected to further investigation, and the data are presented in this communication. There was ample evidence that a qualitative difference occurs in the physical nature of visibles as compared to lethals, and that the ratio between them for the same mutagen was constant and independent of dose and cell stage. Variation in the visible to lethal ratio between agents, therefore, must be a genuine manifestation of differential mutability.

MATERIAL AND TECHNIQUE

All experiments were undertaken with the Oregon-K stock of *Drosophila melanogaster*. This stock has been maintained in our laboratory for many years, and gives a spontaneous sex-linked recessive mutation rate of 0.18 ± 0.05 percent lethals and 0.009 ± 0.001 percent visibles. In control experiments where the males were injected with isotonic saline (0.4 percent NaCl), the spontaneous mutation rate was unchanged.

The compounds to be considered in this communication are the mustard derivatives of amino acids, carboxylic acids, and amines, whose mutagenicity as regards the sex-linked recessive lethals has already been given (FAHMY and FAHMY 1959b). For ease of reference, the formulae and the CHESTER BEATTY (CB) code numbers for these compounds will be restated here in an abbreviated form.

Phenyl amino acid mustards:

$(ClCH_2CH_2)_2N.C_6H_4.(CH_2)_nCOOH$

The derivatives of phenylalanine (n = 1): the L-isomer CB.3025, the D-isomer CB.3026, and the DL-mixture CB.3007 and phenoxyphenylalanine, CB.3051, (n = 1, and the central part of the molecule = C_6H_4–O–C_6H_4).

Phenylcarboxylic acid mustards:

$(ClCH_2CH_2)_2N.C_6H_4.(CH_2)_nCOOH$

The derivatives of acetic acid CB.1331 (n = 1), propionic acid CB.1332 (n = 2), butyric acid CB.1348 (n = 3) and valeric acid CB.1356 (n = 4).

Phenylethylamine mustard:

CB.3034: $(ClCH_2CH_2)_2N.C_6H_4.(CH_2)_2NH_2$

All these compounds have been administered in solution (as sodium salts, except for CB.3034 which was given as the hydrochloride) by our microinjection technique around the testes of adult males. The progeny of the treated males was fractionated (usually at three day intervals) and the mutation rate for the individual broods was determined separately by the Muller-5 technique. The mutations considered in this communication are the sex-linked recessive visibles, scored according to our previously described technique (FAHMY and FAHMY

1955b). The F_2 mutant cultures were scrutinized throughout the full period of adult emergence. Where males of the Muller-5 genotype (*sc^{s1}B In-S w^a sc^s*/Y), and none of the potential wild type class occurred, this was taken to indicate the induction of an X chromosome lethal. Where the potential wild type class was represented by males with anomalous phenotypes, this was taken as an indication of the induction of a sex-linked recessive visible. Only mutations which were confirmed in the F_3 and all subsequent generations were considered in this analysis. Allelism tests were undertaken with the induced visibles against all the known mutants of relevant phenotype (both the α and β loci—see FAHMY and FAHMY 1959a).

A sample of the unidentified visibles, and the recessive lethals, were genetically placed by crossover experiments against a chromosome marked by the genes scute (*sc*), cut (*ct*), vermilion (*v*), forked (*f*) and carnation (*car*). A sample of the localized lethals was analysed cytologically for the detection of aberrations in the salivary gland chromosomes. All preparations were permanent aceto-orcein squashes, sometimes restrained in Feulgen.

OBSERVATIONS

Visible to lethal ratio: One of the most informative techniques in the study of comparative mutagenesis is the analysis of the relative frequency of different mutant types induced in the same treated cells, or better still in the same treated chromosomes. The Muller-5 method for detecting sex-linked recessive mutations is advantageous in this respect, since it enables the determination of both lethal and visible mutations induced in the same sample of treated X chromosomes. A serious difficulty in the classification of these two types of mutation, however, arises from the fact that some 'visible' mutants are also accompanied by reduced viability. It was, therefore, essential to draw a sharp and clear-cut distinction between visibles and lethals, if any use of their relative induction by different mutagens is to be utilized. In our laboratory, therefore, we confine the lethal class to mutations that exert complete killing in the hemizygous state, leaving the visible class to include morphological mutants irrespective of viability.

When the classification of the sex-linked mutations was undertaken on the above basis, it was possible to show (FAHMY and FAHMY 1955b) that the ratio of visibles to lethals induced in the X chromosome by TEM (i.e., triethylenemelamine, or more accurately, 2:4:6–tri(ethyleneimino)–1:3:5–triazine) is reasonably constant and independent of dose and cell stage. This result has now been repeatedly confirmed with several alkylating compounds and is well illustrated by the present extensive data with the mustard derivative of phenylalanine. The effect of the cell stage on the ratio of visibles to lethals cannot be reliably analysed statistically for individual broods. The number of visibles per brood in a manageable experiment is rather small. This could magnify the inevitable error due to the missing of a few mutants because of the generally recognized difficulty in scoring small morphological changes. The problem, however, can be approached indirectly—through the analysis of the over-all ratio in large mutant

samples derived from experiments involving different germ cell stages. If the relative frequency of the two classes of mutation is independent of the cell stage, the over-all ratio between them should be reasonably homogeneous, irrespective of the fraction of the germ line from which the mutations were recovered. Table 1 summarizes the over-all ratio of visibles to lethals in 28 separate experiments undertaken with the phenylalanine mustard. It is to be noted that the experiments were with different doses of the two isomers and their mixture, and they gave widely different lethal rates. Moreover, the mutants were detected in offspring recovered at various times after treatment (0–21 days) and must have been induced in different stages of spermatogenesis. In the greatest majority of the experiments, however, the progeny tested was recovered within the first 12 days, and evidence was available that during this period sperm derived mainly from the postmeiotic stages of the germ line are utilized. Within the last fraction of this period (10–12 days) a total of 33 visibles was detected, but none were in clusters. In contrast, within the following fraction (13–15 days), there were only eight visibles consisting of two small clusters (a pair each) and four single mutants. This indicates that the premeiotic stages are not appreciably utilized earlier than 13 days after treatment. In experiments extending to only 12 days, therefore, no complication due to germinal selection against the lethals occurs, since this process is ineffective among the postmeiotic sperm. Some experiments (extending beyond 12 days) contributed mutations induced in premeiotic stages, where germinal selection could operate. The cytotoxic (killing) effect of the phenylalanine mustard on the premeiotic stages (FAHMY and FAHMY 1959b), however, meant that the contribution from these cells to the over-all mutation pool was extremely small (2.5 percent of the total mutations). In this series of experiments, therefore, the effect of germinal selection can be safely ignored.

The relative frequency of visibles to lethals in the different experiments given in Table 1, has been subjected to extensive statistical analysis by the contingency table technique. The ratio of the two types of mutation was homogeneous for the L-isomer ($\chi^2 = 12.39$, degrees of freedom = 13, $P = 0.5$), as well as for the D-isomer ($\chi^2 = 2.26$, degrees of freedom = 5, $P = 0.8$), but a slight heterogeneity occurred with the DL-mixture, ($\chi^2 = 14.39$, degrees of freedom = 7, $P \simeq 0.05$). The heterogeneity was traced to three anomalous experiments (21, 22 and 27, Table 1) which were undertaken with a faulty sample of the compound. This sample was the first attempt at the conversion of the amino acid mustard to its sodium salt and contained an excess alkali (NaOH) as indicated by its excessively high pH. Biologically, also, this sample gave high toxicity, and abnormally low lethal rates. It is highly probable that this faulty sample was inactivated, in part at least, by the hydrolysis of some of its chloroethyl groups. The excess free alkali could also have affected the biological mode of action. It is of interest to note, however, that the few anomalous experiments did not conceal the over-all homogeneity of the ratio of visibles to lethals on the basis of all experiments ($\chi^2 = 33.14$, degrees of freedom = 27, $P \simeq 0.2$). A comparison has also been undertaken of the relative yield of visibles to lethals in

TABLE 1

Ratio of sex-linked recessive visibles to lethals induced by the mustard derivatives of phenylalanine at different concentrations and in various stages of spermatogenesis

Experiment No.	Dose ($\times 10^{-2}$M)	Days after treatment	Lethal* rate percent	Chrom. tested	Visibles v	Lethals l	Ratio v/l
L-isomer, CB.3025							
1	0.9	0–3	5.0	515	10	26	0.38
2	1.07	0–9	5.4	167	2	9	0.22
3	0.9	0–9	5.5	692	9	38	0.24
4	0.9	0–9	5.8	928	12	54	0.22
5	0.8	0–12	6.0	1,763	36	105	0.34
6	0.9	0–9	6.3	679	16	43	0.37
7	1.07	0–15	6.5	1,177	34	77	0.44
8	1.2	0–18	7.0	1,591	46	112	0.41
9	0.9	0–9	7.7	754	25	58	0.43
10	1.5	0–6	8.4	345	14	29	0.48
11	0.9	0–9	8.5	707	24	60	0.40
12	0.9	0–9	8.9	1,438	29	128	0.23
13	1.07	0–12	9.9	992	35	98	0.36
14	1.07	0–3	14.0	235	12	33	0.36
Total				11,983	304	870	0.35
D-isomer, CB.3026							
15	0.8	0–12	4.6	1,599	21	73	0.29
16	1.07	0–9	7.6	1,023	20	78	0.26
17	0.9	0–6	8.0	399	9	32	0.28
18	1.07	0–9	9.0	927	24	83	0.29
19	1.5	0–6	10.6	198	9	21	0.43
20	1.2	0–9	13.4	179	4	24	0.17
Total				4,325	87	311	0.28
DL-mixture, CB.3007							
21	0.8	0–9	1.7	1,147	4	20	0.20
22	0.51	0–12	2.0	1,793	3	35	0.09
23	0.45	0–21	3.0	2,111	19	64	0.30
24	0.9	0–18	6.1	1,916	43	116	0.37
25	1.2	0–21	6.1	1,851	38	112	0.34
26	1.2	0–12	7.0	660	18	46	0.39
27	1.5	0–6	8.4	885	10	74	0.14
28	1.5	0–9	12.4	322	10	40	0.25
Total				10,685	145	507	0.29
Grand total				26,993	536	1,688	0.32

* This estimate involves the assumption that mitotic proliferation in spermatogonia does not alter the ratio of normal to mutant chromosomes.

the over-all pool of mutants under the L-isomer, the D-isomer and the DL-mixture and no significant difference was detected ($\chi^2 = 4.44$, degrees of freedom $= 2$, $P > 0.1$). There is enough justification, therefore, to utilize the data from all isomers for an accurate determination of the ratio of visibles to lethals for the phenylalanine mustard. The mean value for this ratio is 0.32, with a

standard deviation as estimated from the data of 0.02, which confirms, our earlier estimate (FAHMY and FAHMY 1956).

According to our scheme of classification, morphological mutants, irrespective of viability, are considered as "visibles." A small proportion of these were of low viability and would perhaps be classified by other workers as semilethal. It was thought appropriate, therefore, to test how far this difference in classification could account for the high ratio of visibles to lethals observed under the effect of the amino acid mustard. An analysis of the relative frequency of mutant to Muller-5 males in all the F_2 cultures from which the visibles were obtained is given in Table 2. It can be seen that only nine percent of the observed visibles had a viability less than ten percent that of the Muller-5 males in the same cultures, and might be classified as semilethal. Transferring this proportion from the visible to the lethal class, gives an over-all relative frequency of 486:1738, a ratio of 0.28, which is still not significantly different from the over-all ratio ascertained by our more accurate method of scoring ($\chi^2 = 3.18$, for one degree of freedom, $P = 0.08$).

The relative yield of visibles to lethals under the various other nitrogen mustards investigated are given in Table 3. This yield for another amino acid mustard, *viz.* the phenyoxyphenylalanine compound (CB.3051), is not significantly different from the over-all ratio for the nonsubstituted phenylalanine derivative ($\chi^2 = 0.20$, for one degree of freedom, $P > 0.5$). Under the four carboxylic acid mustards the visible to lethal ratio is extremely consistent ($\chi^2 = 1.55$, degrees of freedom = 3, $P > 0.5$) and is of the order of one visible to ten lethals. The amine mustard gives an over-all ratio of 0.17 which is significantly higher than that for the carboxylic acid mustards ($\chi^2 = 8.02$ for one degree of freedom, $P<0.01$) but is grossly lower than that for the amino acid mustards (compared with the phenylalanine ratio, $\chi^2 = 18.09$ for one degree of freedom, $P < 0.001$).

Among the nitrogen mustards, therefore, the phenylalanine derivative is clearly the most effective agent in the induction of visibles relative to lethals. It is also more efficient in this respect than X-radiation. In experiments with the physical agent comparable to those undertaken by the mustard, as regards experimental conditions and method of scoring, there were two visibles to ten lethals, a ratio which was shown to be significantly lower than that for the amino acid mustard (FAHMY and FAHMY 1957a). More irradiation experiments both with X-rays and γ-rays (from a cobalt^{60} source) have now been undertaken,

TABLE 2

Viability of the hemizygous "visible" mutants relative to the Muller-5 males in the same F_2 cultures

Percent viability	100	99–50	49–10	< 10	Total
No. of visibles	194	134	158	50	536
Percent of total	36.2	25.0	29.5	9.3	100.0

TABLE 3

Ratio of sex-linked recessive visibles to lethals induced by the mustard derivatives of an amino acid CB.3051, and amine CB.3034, and various carboxylic acids, CB.1348, 1331, 1356 and 1332

Experiment No.	Dose ($\times 10^{-2}$M)	Days after treatment	Lethal rate percent	Chrom. tested	Visibles v	Lethals l	Ratio v/l
CB.3051							
1	1.2	0–12	2.6	2,059	15	54	0.28
CB.3034							
1	0.19	0–21	3.2	3,268	20	105	0.19
2	0.29	0–21	4.0	3,271	19	130	0.15
3	0.38	0–21	5.4	1,660	16	89	0.18
4	0.77	0–12	11.4	211	4	24	0.17
5	1.15	0–12	19.1	68	3	13	0.23
Total				8,478	62	361	0.17
CB.1348							
1	0.2	0–12	3.0	1,665	4	50	0.08
2	0.6	0–12	7.4	653	5	48	0.10
3	0.6	0–12	5.6	1,128	4	63	0.06
4	1.0	0–12	13.0	453	4	59	0.07
Total				3,899	17	220	0.08
CB.1331							
1	0.3	0–6	3.2	620	3	20	0.15
2	0.7	0–3	3.6	358	2	14	0.14
3	0.7	0–3	4.6	261	..	12	...
4	1.0	0–3	6.5	309	2	20	0.10
Total				1,548	7	66	0.11
CB.1356							
1	0.5	0–12	1.9	1,504	4	29	0.14
2	0.7	0–12	4.3	1,220	5	52	0.10
3	1.0	0–18	3.0	1,706	7	52	0.13
Total				4,430	16	133	0.12
CB.1332							
1	0.3	0–12	1.5	1,105	2	17	0.12
2	1.2	0–3	2.0	294	..	6	...
3	1.6	0–3	3.9	258	1	10	0.10
Total				1,657	3	33	0.09

and it was possible to confirm the homogeneity of the above ratio in different experiments and its independence of dose and cell stage. In nine radiation experiments there was an over-all of 200 visibles to 978 lethals which when compared to the over-all for the phenylalanine mustard (last row, Table 1) proved to be very significantly lower ($\chi^2 = 22.98$ for one degree of freedom, $P < 0.001$).

The physical nature of the lethals: It is now firmly established that recessive lethals induced by the alkylating mutagens are associated with a lower frequency

of major chromosome aberrations than those induced at the same over-all rate by X-radiation (SLIZYNSKA and SLIZYNSKI 1947; BIRD and FAHMY 1953; FAHMY and BIRD 1953; FAHMY and FAHMY 1956). Even under the polyethyleneimine (TEM), which was shown to be more efficient than X-rays in the induction of chromosome breaks (FAHMY and FAHMY 1954, 1957b), a high proportion of these breaks was eliminated as dominant lethals, leaving a lower rate among viable sperm (FAHMY and FAHMY 1955a) and recessive lethals. On the other hand, the rate of induction of small aberrations (deficiencies and small deletions) under the alkylating mutagens was nearly double that occurring among X-ray lethals induced at the same over-all rate (mainly, FAHMY and FAHMY 1956, 1957b). Formaldehyde-food was also shown (SLIZYNSKA 1957) to induce a high frequency of moderate size deletions relative to other major rearrangements in the salivary chromosomes of the F_1 viable larvae. It is not known, however, whether this efficiency in the induction of deletions could be extended to the smaller deficiencies (five bands or less), nor to what extent these aberrations are recovered among the formaldehyde recessive lethals.

Differences occurred among the alkylating mutagens investigated as regards the proportion of lethals with major chromosome aberrations produced at mutagenically equivalent doses (FAHMY and FAHMY 1956). The most pronounced difference in this connection occurred between the polyethyleneimine (TEM) and the phenylalanine mustard. In lethal samples produced at practically the same over-all rate (8–10 percent), there were 20 percent gross rearrangements under TEM, as compared to two percent under the amino acid mustard. It was, therefore, of interest to determine whether comparable differences occur between mutagens of closer molecular structure. The cytological analysis was accordingly extended to a sample of lethals induced by the most active of the carboxylic acid mustards (p-NN-di-2-(chloroethyl)aminophenylbutyric acid), and the results were compared with those for the amino acid derivative (Table 4). The proportion of major structural rearrangements is significantly higher under the carboxylic mustard (χ^2 with continuity correction $= 3.85$ for one degree of freedom, $P = 0.05$). The frequency of the deficiencies, on the other hand, is approximately the same for the two mustards.

TABLE 4

Chromosome aberrations among sex-linked recessive lethals induced at roughly the same rate (8–10 percent) by the "mustard" derivatives of phenylalanine and phenylbutyric acid

Mutagen	Stocks examined	Gross rearrangements					Deficiencies		Total stocks with chromosome aberrations	
		Trans.	Inv.	Del.	Total No.	Total %	No.	%	No.	%
Phenylalanine mustard	134			3	3	2.2	50	37.3	53	39.6
Phenylbutyric acid mustard	48	1	1	3	5	10.4	17	35.4	22	45.8

This is further evidence in favor of the hypothesis (FAHMY and FAHMY 1957b) that the induction of deficiencies by the alkylating agents is independent of the process of chromosome breakage, and is most probably due to gene inactivation *in situ*.

The loci of induction of recessive lethals along the X chromosome do not seem to differ under the mustard derivatives of phenylalanine as compared to the phenylbutyric acid. The distribution of the genetically placed lethals under these two agents and between the five localization markers are given in Table 5, and it is clear that they are almost identical ($x^2 = 0.40$, degrees of freedom = 4, $P > 0.98$). The distributions within shorter segments (three crossover units groupings) are given in Table 6. The small numbers in the individual cells necessitated the application of the ψ test (SMITH 1952) for the analysis of the correspondence of the two distributions and again revealed no significant difference ($P = 0.74$).

The cytologically detectable loci of chromosome damage (deficiencies and breaks) among the sex-linked recessive lethals induced by the phenylbutyric acid mustard were localized within the lettered subunits of the salivary X chromosome, and their distribution was compared with those of the phenylalanine mustard. The small lethal sample analysed for the phenylbutyric acid derivative, naturally meant a small number of detectable loci of damage and necessitated gross pooling for the statistical test. Table 7 shows the relative frequency of these loci for the two agents, pooled between the markers used in the genetical placings. The application of ψ test to the above data revealed no significant difference in distribution ($P = 0.54$).

In previous studies (FAHMY and FAHMY 1956) we have also demonstrated that no major differences occur in the distribution of the lethals, as well as the larger loci of chromosome damage, under the effect of the phenylalanine mustard, diepoxybutane and a polyethyleneimine (TEM). It would seem, there-

TABLE 5

The distribution of the sex-linked recessive lethals induced by the mustard derivatives of phenylalanine and phenylbutyric acid between the markers of the genetical localization

Segment	Phenylalanine mustard	Phenylbutyric acid mustard	Total	x^2
sc				
0 –19.9	67	14	81	0.026
ct				
20 –32.9	42	8	50	0.133
v				
33 –56.6	73	18	91	0.201
f				
56.7–62.4	12	3	15	0.004
car				
62.5+	25	5	30	0.035
Total	219	48	267	0.399

TABLE 6

The distribution of the sex-linked recessive lethals induced by the mustard derivatives of phenylalanine and phenylbutyric acid among 24 segments of three crossover units each

	Genetical map of the X chromosome																							
Mutagen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total
Phenylalanine mustard	37	8	1	5	9	4	8	11	5	13	8	8	10	4	9	3	7	19	19	1	11	4	10	5	219
Phenylbutyric acid mustard	10		1		1	1	1	2		3	3	2	2		1	1	2	8	2	2	1	2	2	1	48
Total	47	8	2	5	10	5	9	13	5	16	11	10	12	4	10	4	9	27	21	3	12	6	12	6	267

TABLE 7

The distribution of the loci of chromosome damage (breaks and deficiencies) among the sex-linked recessive lethals induced by the mustard derivatives of phenylalanine and phenylbutyric acid among the chromosome segments between the markers of the genetical localization

Genetical map	*sc* 0–19.9	*ct* 20–32.9	*v* 33.0–56.6	*f* 56.7–62.4	*car* 62.5 +	Total
Cytological map	$1A–7B_{1.2}$	$7B_3–9F_{13}$	$10A_{1.2}–15E_7$	$15F_{1.2}–19A_{5.6}$	$19B_{1.2}$–centromere	
No. of bands	301	178	343	157	49	1,028
Loci of damage						
phenylalanine mustard	39	25	45	6	23	138
phenylbutyric acid mustard	6	4	7	1	8	26

fore, that the loci of lethal induction along the X chromosome are essentially the same under the effect of various alkylating mutagens.

The sites of induction of visibles and lethals: On the basis of the view that visibles and lethals are different phenotypic expressions of the same mutations, no differences in their sites of induction would be expected to occur. It has now been ascertained that the distribution of the lethals along the X chromosome is essentially the same under different alkylating mutagens. If the assumption of physical identity of the two mutant types is to be satisfied, the distribution of visibles should also be comparable among various alkylating mutagens and similar to that of the lethals, at least for the same agent. However, it has already been shown (FAHMY and FAHMY 1959a) that differences do occur in the relative distribution of visible alleles under different alkylating mutagens. There now remains to determine the relationship of the distributions of the visibles relative to the lethals under the same compound.

The data for the phenylalanine mustard are sufficiently extensive for the examination of the above problem. But, before any reliance can be placed on the relative distribution of the two mutant types, it is essential to determine how consistent is the distribution of each separately, particularly under different doses. In order to guard against the possible artifacts of placing in the linkage experiments, we confined the analysis to the distribution of the mutants among the five segments between the localization markers (*sc-ct-v-f-car*-centromere). The placed lethals (Table 8) were recovered from three concentrations: 1.5, 0.9

TABLE 8

The distribution of the sex-linked recessive lethals induced by the mustard derivative of phenylalanine between the localization markers, under different concentrations. (The expected numbers are given in brackets)

Segment	Concentration ($\times 10^{-2}$M) 1.5	0.9	0.8	Total
sc				
0 –19.9	22	18	27	67
	(23.2511)	(18.9680)	(24.7808)	
ct				
20 –32.9	19	11	12	42
	(14.5753)	(11.8904)	(15.5342)	
v				
33 –56.6	26	20	27	73
	(25.3333)	(20.6667)	(27.0000)	
f				
56.7–62.4	1	5	6	12
	(4.1644)	(3.3973)	(4.4384)	
car				
62.5+	8	8	9	25
	(8.6758)	(7.0776)	(9.2466)	
Total	76	62	81	219

$X^2_{[8]}=6.46$; $P > 0.5$.

and 0.8 ($\times 10^{-2}$M), and the numbers occurring in the various segments were reasonably adequate for the statistical analysis. The number of placed visibles from the above experiments, on the other hand, was much too small for the statistical tests, but a great many visibles were placed from other experiments. In order to make full use of all the data, and thus make the analysis more reliable, pooling of visibles from experiments at close doses was necessary. This was undertaken to correspond most closely to the concentrations inducing the lethals and in such a way as not to prejudice the possibility that lethal induction may be favored by stronger treatment. The experiments were, therefore, classified into three groups with the concentration ranges: 1.5–1.2; 1.1–0.9 and 0.8–0.5 ($\times 10^{-2}$M), and these were considered to be equivalent to the three doses inducing the lethals. The over-all visibles falling in the five marked segments for the three dose ranges are given in Table 9. The lethal data in Table 8 were subjected to a 3×5 contingency test and revealed complete homogeneity ($\chi^2 = 6.46$, degrees of freedom = 8, $P > 0.5$). The same analysis was applied to the visible data (Table 9) and also revealed no significant heterogeneity ($\chi^2 = 13.28$, degrees of freedom = 8, $P \backsimeq 0.1$). It is, therefore, certain that the distribution of the lethals by themselves, and the visibles by themselves, among the five marked segments of the X chromosome are homogeneous and independent of the strength of the treatment. This distribution for each category of mutations, therefore, must be a function of the compound.

TABLE 9

The distribution of the sex-linked recessive visibles induced by the mustard derivative of phenylalanine, between the localization markers, under different concentrations. (The expected numbers are given in brackets)

Segment	Concentration ($\times 10^{-2}$M) 1.5–1.2	1.1–0.9	0.8–0.5	Total
sc				
0 –19.9	36 (48.3852)	71 (66.2667)	35 (27.3481)	142
ct				
20 –32.9	25 (22.1481)	27 (30.3333)	13 (12.5185)	65
v				
33 –56.6	52 (46.3407)	66 (63.4667)	18 (26.1926)	136
f				
56.7–62.4	19 (13.9704)	14 (19.1333)	8 (7.8963)	41
car				
62.5+	6 (7.1556)	11 (9.8000)	4 (4.0444)	21
Total	138	189	78	405

$\chi^2_{[8]} = 13.28$; $P \backsimeq 0.1$.

The lethal and visible data (Tables 8 and 9) were then statistically analysed together, to determine whether the distributions of the two mutant types differ relative to one another. This analysis was undertaken by YATES' (1955) recent technique for the combination of probabilities from a set of 2×2 tables. The frequency of mutants within each segment to those in the rest of the chromosome (for the lethals as well as the visibles at each dose) was compared in a 2×2 table, and a χ^2 for the deviation was determined. In this study, the direction, as well as the magnitude of the deviation, is important. χ, (i.e., $\sqrt{\chi^2}$), was therefore calculated with sign: + ve when the proportion of lethals was higher, and − ve when the excess was in favor of the visibles. In the combination test from all doses, the values of χ were algebraically summed, before the assessment of the significance of the overall trend. The summary of the statistical analysis is given in Table 10. A decisive heterogeneity in the relative distribution of the two classes of mutation occurs in the last two segments of the chromosome. A disproportionate excess of visibles is induced between *f-car*, whereas relatively more lethals occur beyond *car*.

The excess lethals in the proximal chromosome segment (*car*–centromere) is indeed most impressive and consistent. The experiments at the various dose levels show the deviation in the same direction (in favor of the lethals) and to roughly the same degree. This strongly suggests that the relative induction of lethals to visibles is independent of the strength of the treatment, at least within the dose range utilized (roughly threefold). In view of the narrowness of this range, however, it was thought appropriate to subject the above conclusion to a more stringent test, in an indirect way. If lethals are simply the more severe counterparts of visibles, then by comparing the proportion of lethals at the low doses with that of the visibles at the high doses, the difference should be concealed, or, at least, its magnitude reduced. The same applies when comparing the lethal proportion at the high doses with that of the visibles at the low doses. We, therefore, applied this double switch for the proportion of the lethals and visibles in the proximal

TABLE 10

The distribution of χ for the relative proportions of lethals and visibles induced by the mustard derivative of phenylalanine, between the localization markers, under different concentrations

Segment	Concentration ($\times 10^{-2}$M) 1.5–1.2	1.1–0.9	0.8–0.5	Total χ	No. of estimations	P
sc						
0 –19.9	0.4510	−1.2207	−1.4900	−2.2597	3	0.10
ct						
20 –32.9	1.1916	0.6589	−0.3206	1.5299	3	0.19
v						
33 –56.6	−0.5049	−0.3833	1.4353	0.5471	3	0.37
f						
56.7–62.4	−2.9950	0.1697	−0.6338	−3.4591	3	0.02
car						
62.5+	1.7493	1.8303	1.3748	4.9544	3	0.002

segment for the two extreme doses, leaving the data for the medium doses unchanged. With these new combinations, the sum of x for the three estimates increased from 4.95 to 4.99, thus indicating an even greater significance for the relative excess in lethals.

A consideration of the over-all data (from all doses) brings out the phenomenon of differential response within the last two chromosome segments: *f-car* and *car*-centromere, most clearly (Table 11). The proportion of mutants proximal and distal to *f* (i.e., *sc-f* and *f*-centromere) is amazingly comparable for both the visibles and the lethals, giving a rough percentage ratio of 85:15. When the actual data for the distribution of the two classes of mutation between the above segments were compared in a 2×2 table, no significant difference was detectable ($\chi^2 = 0.27$, for one degree of freedom, $P \simeq 0.6$). However, on examining the distribution of lethals and visibles between the two marked subsections proximal to *f* (i.e., *f-car* and *car*-centromere), a very major difference becomes immediately discernible. The lethal proportion is twice as high beyond, as before, *car* (5.5:11.4 percent), while the visibles show the reverse proportions (10.1:5.2 percent) within the same segments. When only the mutants that occurred beyond *f* were considered, and the relative frequencies of the visibles and lethals on either side of *car* were compared in a 2×2 table, a very significant difference was detected ($\chi^2 = 10.58$, for one degree of freedom, $P \simeq 0.001$). There can hardly be any doubt, therefore, that a differential induction of visibles to lethals occurs proximal to *f*, with a relative excess of visibles in the chromosome segment *f-car*, and excess lethals in *car*-centromere.

DISCUSSION

The fascinating discovery of differential gene response to mutagens has already been discussed in detail (FAHMY and FAHMY 1959a). The phenomenon is

TABLE 11

The over-all distribution of the sex-linked recessive visibles and lethals induced by the mustard derivative of phenylalanine, showing the differential yield proximal to forked

Segment	Visibles v	Lethals l	Total	Proportion (percent) v	Proportion (percent) l	P [χ^2]	
sc 0 –56.6	343	182	525	84.7	83.1		
f 56.7–62.4	41	12	53	10.1	5.5	0.001	0.6
car 62.5+	21	25	46	5.2	11.4		
f–centromere 56.7+	62	37	99	15.3	16.9		
Total	405	219	624	100.0	100.0		

amply manifested in the present data, by the variation of the ratio of visibles to lethals induced in the X chromosome even under chemically related mutagens. Foremost in this connection is the observation that the mustard derivative of phenylalanine induces three visibles to ten lethals, which was found to be significantly higher than the ratio of 1:10 induced by the majority of the other difunctional alkylating mutagens (FAHMY and FAHMY 1956, 1958a) and also higher than the ratio of 2:10 occurring with radiation. It cannot be overemphasized, however, that these ratios are mean values, based on large scale experiments. Particularly in the case of the phenylalanine mustard, consideration was given to variation due to optical isomerism, dose and cell stage, as well as to the uncontrollable experimental errors. As was demonstrated (Table 1), the mean value of this ratio is 0.32, with a standard deviation as estimated from the experimental data of $\pm$ 0.02.

In contrast to our data on the phenylalanine mustard, there is the ALTENBURGS' (1959) single experiment yielding 27 lethals (with three presumed 'clusters') and no visibles, whereas on the basis of our mean ratio (3:10) a minimum of six visibles would be expected. Statistically such a deviation is significant. It is to be noted, however, that the above expectation has been estimated on the basis of the binomial distribution and completely ignores the experimental variation, which could be quite substantial due to difficulty in scoring visibles. Obviously the ALTENBURGS' single experiment cannot supply the range of their experimental variation, thus making their statistical test of little biological value. In our experiments, it is certain that the experimental error did not play a major role, as indicated by the small standard deviation of the mean as estimated from the whole data. Nevertheless, one of our atypical experiments (experiment 22, Table 1), which is most comparable in size and lethal rate to the ALTENBURGS', gave an anomalous ratio of 0.09, on the basis of which the absence of visibles in an experiment giving 27 lethals would be insignificant. This illustrates the danger of basing any conclusions as regards the induction of visibles on a single experiment with a low mutation rate. It is perhaps suggestive that the anomalous visible to lethal ratios in our experiments occurred with a faulty sample of the racemic mixture, which was partly inactivated by hydrolysis. This may indicate that the quality and purity of the ALTENBURGS' compound may have been substandard. Whatever the reason for the discrepancy of the visibles in these authors' experiment, it is highly unlikely to be the different method of scoring developed in our laboratory (FAHMY and FAHMY 1955b, see also MATERIAL AND TECHNIQUE). As already stressed (see OBSERVATIONS), even when visibles of low viability were classified as full lethals, the ratio of visibles to lethals under the phenylalanine mustard was not substantially decreased below the postulated 3:10 mean.

The significance of the variation in the ratio of visibles to lethals under different mutagens, has recently been discussed, on purely theoretical grounds, by both AUERBACH (1958) and the ALTENBURGS (1959). To these authors the classification of mutations into visibles and lethals is merely a matter of con-

venience, which is "neither strict nor biologically very meaningful" (AUERBACH 1958). They maintain that a "lethal" is a "visible" of very low viability. As a phenotypic description of the two types of mutation, this is perhaps tenable, since there is a continuous spectrum of viability from visibles through semilethals (including some visibles) to full lethals. This is, indeed, the reason why in our laboratory we confine the lethal class to mutations that exert *complete* killing in the hemizygous state, leaving the visible class to include morphological mutants irrespective of viability. In spite of the "merging" in the viability expression of the two mutant classes, however, it has long been known that differences do occur in their physical nature (as manifested in the salivary chromosomes). Lethals are often associated with chromosome aberrations, while visibles are mostly point mutations unaccompanied by such changes. This has been amply illustrated in the radiation mutagenesis work. In a sample of recessive lethals and visibles induced at specific X chromosome loci in the same series of X-ray experiments (DEMEREC 1937), nearly 40 percent of the lethals were shown to occur at one of the breakage points of major chromosome rearrangements, while none of the visibles were caused by this mechanism. Furthermore, viable "visible" changes in the white-Notch region (DEMEREC 1939) were not only devoid of major chromosome rearrangements, but also unassociated with small deficiencies which are known to occur most frequently in this region. The radiation mutability results at specific loci on the autosomes (third chromosome, WARD and ALEXANDER 1957) are closely parallel to the earlier results with the X chromosome. The fully viable visibles gave a percentage ratio of cytologically detectable aberrations to apparently normal of 20:80, while this ratio for the lethals was 60:40. Extensive mutability tests at various X chromosome loci under the effect of the phenylalanine mustard have now been undertaken (FAHMY and FAHMY 1959a), and the cytological analysis of the mutants produced is still in progress. Nevertheless, all the fully viable visibles so far observed proved to be point mutations, whereas the lethals were associated with deficiencies or major aberrations.

The above considerations show that the viability scale of the visible-lethal mutants induced in a given chromosome sample does bear some relationship to the physical nature of the genetic damage induced. The scale devised in our laboratory is to refer the frequency of the morphological mutants to those which produce extreme depression of viability so as to result in complete elimination of the hemizygous adults. If, on the basis of this classification, two mutagens induce different relative frequencies of the two classes of mutation, it would seem reasonable to infer that their mode of action on the hereditary material must be somehow different. The question then arises as to the exact mechanism responsible for this difference. The apparent association between chromosome breakage and recessive lethals led to the conclusion (mainly CATCHESIDE 1948) that these mutations are indeed breaks which either restitute or undergo exchange. Visibles, on the other hand, are assumed to be intragenic changes. On this basis, the visible to lethal ratio will be highest for mutagens with the lowest potency in chromo-

some breakage. The amino acid mustards are, indeed, the weakest breakers and they do give the highest visible to lethal ratio (3:10). On the other hand, the greatest majority of the other alkylating mutagens (imines, epoxides and carboxylic acid mustards) give a ratio of one visible to ten lethals, which was found to be lower than that given by radiation (2:10). This is so in spite of the fact that the majority of the above compounds were shown to induce significantly less major rearrangements among viable sperm as well as among recessive lethals than mutagenically equivalent doses of radiation. It would seem, therefore, that the ratio of visibles to lethals is not entirely controlled by the agent's efficiency in the induction of intra- to intergenic mutations though obviously this must be playing a partial role.

Another mechanism which affects the yield of lethals is the mutagen's potency in the induction of small deficiencies (less than one percent of the length of a salivary chromosome arm). Among sex-linked recessive lethals induced at the same over-all rate, the alkylating agents gave roughly the same proportion of small deficiencies which was double that for X-radiation. Yet, differences do occur in the ratio of visibles to lethals among the alkylating mutagens. Therefore, the efficiency in the induction of deficiencies is not the controlling factor in the variation of the visible to lethal ratio.

It is virtually certain, therefore, that no one physical mechanism for the production of lethals can be singled out, as the sole, or even the major, cause in the variation of the relative induction of visibles to lethals by different mutagens. This variation must be a function of the mutagen's ability to induce visibles as a class, relative to lethals as an integral group, irrespective of the latter's detailed constitution as regards chromosome breaks, deficiencies and point mutations. The only mechanism that can embrace the three physical causes for lethal production is that of genetic loss of microscopic or submicroscopic (i.e., molecular) dimensions. The alternatives for the visibles, would be either a lower degree of genetic loss, or an intramolecular reorientation within the genes without any loss of material. The fact that the ratio of visibles to lethals for any one compound is independent of dose (FAHMY and FAHMY 1955b; Present paper, Tables 1 and 3) argues against the possibility that the difference is entirely a matter of degree and favors a genuine qualitative difference between the two mutant classes.

The difference in the nature and loci of induction of visibles and lethals became abundantly clear through the study of their distribution along the X chromosome under the phenylalanine mustard. The distribution of mutants belonging to each class was consistent and independent of dose, but the relative distribution of the two mutant classes was decisively different. The differential response occurred mainly in the proximal part of the X chromosome (*f*-centromere). Within this segment the relative proportions of the two mutant types showed an excess of visibles distal to *car* (*f-car*) and an excess of lethals nearer the centromere (beyond *car*). It was further possible to show that the relative excess of the lethals beyond *car* was again independent of the strength of the

treatment. There is no doubt, therefore, that some gene sites do show a varied susceptibility to the induction of the two classes of mutation.

The excess in the relative yield of lethals compared to visibles close to the centromere (beyond *car*) may perhaps be attributed to its large heterochromatic content. Heterochromatin is relatively inert, and it would, therefore, be conceivable that the proximal segment contains few genes that can affect morphological characters (i.e., visibles). While this possibility cannot be entirely excluded, it does not seem to be supported by the data on the visible loci so far mutated. By radiation and a diversity of alkylating mutagens, an over-all of 73 sex-linked visible loci have so far been mutated beyond *f*. Of these, there were 40 between *f-car*, and 33 beyond *car*—a relative frequency which is not significantly different from equality ($\chi^2 = 0.67$, for one degree of freedom, $P > 0.3$).

Another feature that may be attributed to heterochromatin, is that it may consist of a less rigid variety of nucleoprotein, which is more vulnerable to damage by loss, thus favoring the induction of lethals. This phenomenon could certainly be ascertained by a consideration of the cytological nature of the lethals induced by the phenylalanine mustard (see Observations). The frequency of the loci of chromosome damage (breaks and deficiencies) can be taken as a function of the efficiency of gene elimination, while the number of salivary chromosome bands is perhaps the best estimate of genic content. Within the same sample of lethals analysed (Table 7) there were six loci of damage between *f-car*, compared to 23 beyond *car*, indicating a significant excess in the latter segment ($\chi^2 = 9.97$, for one degree of freedom, $P < 0.01$). This is so in spite of the fact that the physical length of the *f-car* segment includes 157 bands while the segment beyond *car* contains only 49 bands. Futhermore, the number of loci of damage detected beyond *car* is probably an underestimate since a great many rearrangements induced in segment 20 of the salivary X chromosome are apt to be missed because of the confused chromocentre. It is, therefore, certain that the frequency of the loci of damage relative to the gene content is very grossly higher beyond *car* than between *f-car*. The relative excess of lethals to visibles beyond *car*, therefore, could reasonably be attributed to the ease of gene elimination within the heterochromatin.

The excess in the proportion of visibles relative to lethals occurring in the *f-car* segment is indeed of great biological significance. The structure of this segment is exclusively euchromatic, or, at any rate, it is the freest from heterochromatin. This clearly shows that the differential induction of visibles to lethals is by no means restricted to the heterochromatic chromosome segments, but could also occur in the euchromatin. This differential gene response may well be brought about by a mechanism analogous to that inferred for the segment beyond *car*, only in the opposite direction. The genes within the *f-car* segment are probably more susceptible to molecular reorientation, rather than mutation by material loss.

The establishment that lethal and visible mutations are qualitatively different in nature and distribution along the chromosome introduces a problem as to

whether they are to be considered as occurring within the same or different genes. The recent discovery of the composite nature of the gene (BENZER 1956), both as regards structure and function, reduces any distinction between the hereditary units to the level of convenient terminology. Applying BENZER's nomenclature, lethals and visibles must now be looked upon as the manifestations of different mutons, since they could obviously involve different mutational sites. It is not known, however, whether these sites occur within the same or different functional subunits: cistrons, or even whether they belong to the same or different larger units: genes. Theoretically, it is conceivable that the genome of an individual could be differentiated into genes for the vegetative (metabolic) functions controlling viability: mutating to lethals, and others governing morphogenesis: mutating to visibles. In practice, however, it is difficult to be certain that any one gene does either of the above functions to the exclusion of the other. It would, therefore, seem convenient to think of visibles and lethals as different changes within the same genes. The differential yield of visibles to lethals, therefore, could best be visualized as resulting from reactions with various molecular receptors of the genetic material. An attack of a certain type results only in molecular reorientation sufficient to alter the transmission of the genetic code, thus resulting in a visible. Another type of attack is sufficiently different to result in the partial, or complete, elimination (or inactivation) of the gene, thus resulting in a lethal.

SUMMARY

The ratio of visibles to lethals induced in the same sample of treated X chromosomes shows significant variation even among closely related mutagenic compounds. For the same compound, however, this ratio is constant and independent of dose and cell stage. The distribution of each of the mutant classes under the same compound (the phenylalanine mustard) along the X chromosome is also constant and independent of dose. The relative distribution of the two mutant classes, however, is significantly different. Between *f-car*, the proportion of visibles induced is higher than that of the lethals, while beyond *car*, the excess is in the proportion of the lethals. The whole data indicate a qualitative difference in the physical nature of visibles and lethals. A hypothesis is formulated that visibles are the result of intramolecular reorientation within genes while lethals involve material loss on the genic or subgenic level.

ACKNOWLEDGMENTS

We wish to thank PROFESSOR A. HADDOW, F.R.S., for his interest, and our colleagues of the Chemistry Department, particularly PROFESSOR F. BERGEL, F.R.S., DR. W. C. J. ROSS, and DR. J. A. STOCK for the supply of the mutagens and their advice as to their chemical properties.

This investigation has been supported by grants to the Chester Beatty Research Institute from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, The Anna Fuller Fund, and the National Institute of Health, U.S. Public Health Service.

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THE GENETICS OF PAPILIO DARDANUS, BROWN. II. RACES DARDANUS, POLYTROPHUS, MESERES, AND TIBULLUS

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Received September 28, 1959

IN Part I (CLARKE and SHEPPARD 1959) we described the genetics of *P. dardanus* race *cenea* from South Africa. The present paper concerns four other races of the butterfly—*dardanus, polytrophus, meseres* and *tibullus*. The map (Figure 1) shows the distribution of these and their geographical relationships

FIGURE 1.—Distribution and geographical relationship of *P. dardanus* races.

with *P. dardanus cenea*. In addition the areas inhabited by the Madagascan race *meriones* and the Abyssinian race *antinorii* are indicated, and these forms will be the subject of a separate paper (Part III).

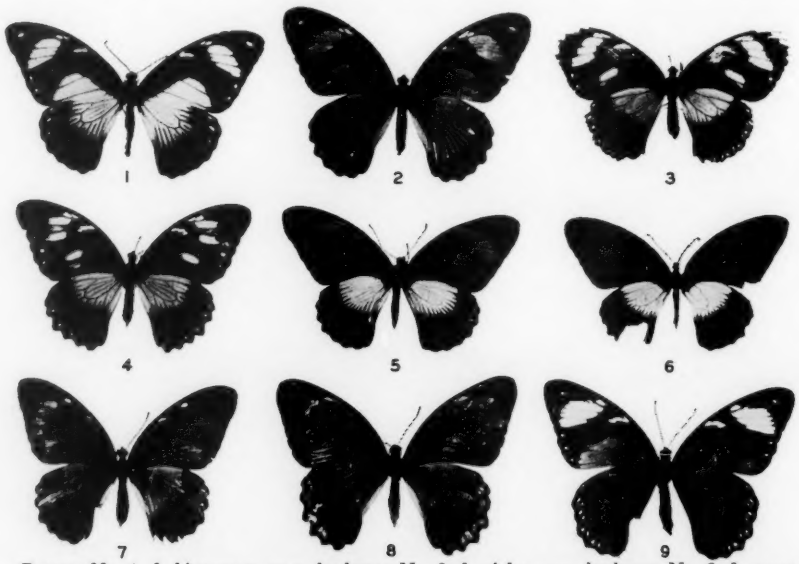
RACE DARDANUS

This has the most extensive range of any of the races of *P. dardanus*. It is found down the West Coast of Africa from Sierra Leone to Angola, and spreads eastwards towards Uganda and Tanganyika Territory to Lake Victoria. In these areas it merges into the transitional race *meseres*. In the western part of its range the female is almost invariably the mimetic form *hippocoon*, and this closely resembles the form *hippocoonides* already described under race *cenea* from South Africa (Part I).

In race *dardanus* we have bred *hippocoon* both from the eastern and western parts of its range and have also investigated f. *trophonissa*, f. *niobe* and f. *planemoides*. The mimetic form *cenea* has not so far appeared in our material but we have carried out a race cross using this form from South Africa (see below).

F. hippocoon

This form (Plate, No. 1) which mimics *Amauris niavius niavius* is very similar to *hippocoonides*, and, wherever we have tested it in relation to other forms,



PLATE—No. 1. f. *hippocoon*, race *dardanus*. No. 2. f. *niobe*, race *dardanus*. No. 3. f. *cenea* showing breakdown of mimicry. No. 4. f. *cenea*, showing no breakdown of mimicry. No. 5. f. *planemoides*, race *dardanus*. No. 6. f. *proto-planemoides*. No. 7. f. *poultoni*, race *polytrophus*. No. 8. f. *dorippoides*. No. 9. f. *trophonissa*, race *dardanus*.

we have found that it behaves similarly to *hippocoonides*. As in Part I, therefore, we have assumed that *hippocoon* and *hippocoonides* and the hybrids between the two are equally satisfactory for testing either for dominance relationships or allelomorphism.

Hybrids between f. hippocoon *and f.* cenea *from South Africa:* It is of interest that when f. *hippocoon* from the West Coast of Africa (where *cenea* does not occur) is crossed with f. *cenea* from South Africa, the mimetic pattern of f. *cenea* is broken down (Plate, No. 3). On the other hand, when f. *hippocoon* from the eastern range of *dardanus* (where f. *cenea* does occur but at a low frequency) is crossed with f. *cenea* from South Africa, the breakdown of the mimetic pattern is very much less (No. 4). This suggests that on the west coast the population does not possess the modifying genes perfecting the mimicry of f. *cenea*, whereas these are present further east. In contrast the mimetic pattern of f. *trophonius* is not appreciably altered when crossed with f. *hippocoon* from race *dardanus*. This we believe to be because f. *trophonissa* is found throughout the range of the race (CLARKE and SHEPPARD 1960 for elaboration of this point).

F. niobe

This is a bright orange insect with an orange body (Plate, No. 2). It has the pattern of *trophonissa*, but with the subapical spot of the forewing orange instead of white. It is therefore much like f. *salaami* from South Africa (see Part I) but the hind wings are more rayed as in *hippocoon*.

Autosomal inheritance of f. niobe: It is clear that the gene controlling this form is, like the others investigated, autosomal. Thus, in brood 3228 a *niobe* female, mated to a male which cannot have carried *niobe*, produced seven *niobe* daughters, showing that the gene cannot be on the X chromosome. That it cannot be sex-linked on the Y chromosome is demonstrated in brood 3029, where a female *hippocoon* which could not have carried *niobe*, when mated to a male which had eleven *niobe* sisters, produced 23 males, ten *hippocoon* females and eight *niobe* females (see Table 1).

TABLE 1

Autosomal inheritance of f. niobe (*race* dardanus)

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Offspring		
			Males	*hippocoon* females	*niobe* females
3029	*hippocoon*	heterozygous *niobe/hippocoon* $H^{Ni}h$*	23	10	8
3228	*niobe* 3029 $H^{Ni}h$	*hh*† (cannot be carrying *niobe*)	9	3	7

* $H^{Ni}h$ = *niobe/hippocoon*.

† The designation of the genotype of the male known to be homozygous for *hippocoonides*, *hippocoon* or the hybrid between the two.

N.B. The full list of the genetic symbols used to indicate the various genotypes in all races of *P. dardanus* will be given at the end of Part III.

Dominance relationships of f. niobe: In brood 3017 a female *niobe* 2779 mated to a male known to be homozygous for *hippocoon(ides)* produced six males, two *hippocoon(ides)* and four *niobe* females. (A *hippocoon* or *hippocoonides* of hybrid origin is written as *hippocoon(ides)* in this paper if race *dardanus* or *antinorii* is involved.) Again in brood 3029 a *hippocoon* female 2862 (in which no *niobe* had appeared) mated to a male 2802 (which had *niobe* among its sibs), produced 23 males, ten *hippocoon(ides)* and eight *niobe* females. Broods 3017 and 3029 therefore prove that f. *niobe* is dominant to f. *hippocoon(ides)* and pure *hippocoon*.

When crossed with f. *cenea* (from South Africa) *niobe* either forms a recognizable heterozygote with *cenea* or else is fully dominant to it. In brood 2779 a *niobe* female 2666 (not carrying *cenea*) mated to a male 2640 heterozygous for *cenea* and *hippocoonides* (and not carrying *niobe*) produced 11 males, two *cenea*, three *hippocoon(ides)*, two *niobe* and two females with *cenea* patterning and *niobe* coloring ($H^{c}H^{Ni}$, the heterozygotes called red-brown *cenea*). In contrast, in brood 2802 a *cenea* female which could not have been carrying *niobe* was mated to a male heterozygous for *niobe* and *hippocoon*. Their offspring were 27 males, six *cenea*, six *hippocoon(ides)* and 11 *niobe* females. No recognizable heterozygotes were produced in this brood. F. *cenea* thus behaves with f. *niobe* much as it does with f. *leighi* (see Part I).

Niobe can also be formed as the heterozygote between f. *trophonius* and f. *planemoides*. In brood 3059 a *trophonius* female heterozygous for *hippocoon(ides)* (and not carrying *niobe*) was mated to a male heterozygous for f. *planemoides* and f. *hippocoon* (the male had five *hippocoon* and five *planemoides* sisters and ten brothers, none of which when mated produced *niobe*). The offspring of brood 3059 were one male, one *hippocoon(ides)*, one *trophonius* and five "synthetic" *niobe* females. We have shown, however, on several occasions that f. *niobe* does not usually arise in this way, and that it is more often inherited as a unit. Thus in brood 3029 a male carrying *niobe* was mated to a *hippocoon* female and produced 23 males, ten *hippocoon(ides)* and eight *niobe* females. Again in brood 3017 a *niobe* female mated to a male homozygous for *hippocoon(ides)* produced six males, two *hippocoon(ides)* and four *niobe* females. Broods 2967 and 3228 give similar information. In none of these instances has *niobe* given rise to *planemoides* or *trophonius* (see Table 2). It is not known how often *niobe* is formed in the wild by a combination of *trophonius* and *planemoides*, but this could well happen since the forms are sympatric in the eastern part of the range of race *dardanus*.

More work on *niobe* is necessary before we have formal proof that it is controlled by a gene at the same locus as the other forms, though this is suggested by our data.

F. trophonissa

This orange insect with a white subapical spot (Plate, No. 9) resembles the mimic f. *trophonius* from South Africa (Part I) except that the upper side of the hindwing has more marked longitudinal rays. In brood 2862 (Table 3) a *tro-*

TABLE 2

Dominance relationships of f. niobe, (*race* dardanus)

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Offspring: Males	*cenea* females	Recognizable heterozygote *niobe/cenea* females	*hippocoon* females	*niobe* females	*trophonius* females	*trophonius/planemoides*, synthetic *niobe* females
2779	*niobe* $H^{Ni}h$	*polytrophus* $H^{c}h$	11	2	2	3	2	0	0
2802	*cenea* $H^{c}h$	$H^{Ni}h$	27	6	0	6	11	0	0
2967	*niobe* 2802 $H^{Ni}h$	*hh*	6	0	0	6	5	0	0
3017	*niobe* 2779 $H^{Ni}h$	*hh*	6	0	0	2	4	0	0
3029	*hippocoon* *hh*	$H^{Ni}h$ 2802	23	0	0	10	8	0	0
3059	*trophonius* $H^{T}h$	$H^{Pl}h$ 2863 see Table 5	1	0	0	1	0	1	5
3228	*niobe* 3029 $H^{Ni}h$	*hh*	9	0	0	3	7	0	0

hh=*hippocoonides*, *hippocoon* or the hybrid. Such an insect of hybrid origin is written *hippocoon(ides)* or *h'(ides)*.
$H^{c}h$=*cenea/hippocoon*.
$H^{Ni}h$=*niobe/hippocoon*.
$H^{T}h$=*trophonius/hippocoon*.
$H^{Pl}h$=*planemoides/hippocoon*.

phonissa female mated to a wild male produced 17 *trophonissa* and 18 *hippocoon* females. If *hippocoon* were dominant to *trophonissa*, all the offspring of 2862 would carry the latter form. In fact this is almost certainly not the case because in broods 3015 and 3040 (Table 3) no butterflies resembling the recognizable *trophonius/planemoides* heterozygote (i.e., synthetic *niobe*) or *trophonissa* appeared. Therefore, *trophonissa*, like *trophonius*, is almost certainly dominant to *hippocoon*. If this is so, brood 3002 (Table 3), where the father is not carrying *trophonissa* shows that *trophonissa* is neither sex-linked on the X nor on the Y chromosome (because *hippocoon(ides)* was produced), but is, like the genes controlling the other forms, autosomal. It seems highly probable that *trophonissa* is a modified *trophonius* and controlled by the same major gene. Thus in race crosses between the South African *trophonius* and *hippocoon*, the hybrids resemble *trophonissa* having the rayed hindwing characteristic of this form and of *hippocoon*.

TABLE 3

Dominance relationships and autosomal inheritance of f. trophonissa (*race* dardanus)

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Offspring			
			Males	*hippocoon* or *h'(ides)* females	*trophonissa* females	*planemoides* females
2862	wild *trophonissa*	wild male race *dardanus*	13	18	17	0
3002	$H^{T}h$ *trophonissa* 2862	*leighi/ hippocoon(ides)* also carrying part of gene complex of *antinorii*, Brood 2876 (see Part III)	20	8	6	4 (2 of these were *proto-planemoides*)
3015	$H^{Pl}h$ *planemoides* 2863 see Table 5	*hh* 2862	15	9	0	4
3040	$H^{Pl}h$ *planemoides* 2863 see Table 5	*hh* 2862	14	13	0	11

$H^{Pl}h$ = *planemoides/hippocoon.*
$H^{T}h$ = *trophonissa/hippocoon.*
hh = *hippocoon.*

F. planemoides

This form has an irregular orange band on the forewing and white hindwings with a wide black border (Plate, No. 5). It is a mimic of *Bematistes poggei.*

Autosomal inheritance of f. planemoides: Broods 3015 and 3040 show that the gene controlling *planemoides* is not sex-linked on the X chromosome, and brood 3005 shows that it is not sex-linked on the Y. The gene therefore is autosomal (Table 4).

Dominance relationships of f. planemoides: In brood 2863, (Table 5) a wild *planemoides* female from Entebbe produced 11 males, five *hippocoon* and five *planemoides* females. A female *planemoides* of brood 2863 mated to a male from brood 2862 (which segregated *trophonissa* and *hippocoon*, Table 3) gave 15 males, nine *hippocoon* and four *planemoides* females (brood 3015, Table 5). Mating 3040 (Table 4) shows a similar situation, and these two broods show that *planemoides* is dominant to *hippocoon.*

Race crosses using f. planemoides: When a female f. *planemoides* from race *dardanus* was crossed with a male known to be homozygous for *hippocoonides* from race *polytrophus* (where the mimicry is imperfect, often due to the presence of male-like yellow scales), the mimetic pattern of *planemoides* was broken down. This occurred in brood 3049 (Table 5) where a *planemoides* female 2863 was mated to a male homozygous for *hippocoonides* with some *polytrophus* but no

TABLE 4

Autosomal inheritance of f. planemoides (*race* dardanus)

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Offspring		
			Males	*hippocoon* females	*planemoides* females
3005	*hippocoonides* unknown brood	$H^{Pl}h$ 2863 wild	8	2	4
3015	*planemoides* 2863 see Table 5	*hh*	15	9	4
3040	*planemoides* 2863 see Table 5	*hh*	14	13	11

$H^{Pl}h$ = *planemoides/hippocoon.*
hh = *hippocoon.*

dardanus in its ancestry. The offspring of mating 3049 were eight males, two *hippocoon(ides)* and three *proto-planemoides* females. In these the normal orange band on the forewing was interrupted by a horizontal black bar (Plate, No. 6). (The prefix *proto-* signifies that the form concerned is an imperfect mimic).

As with form *niobe*, "synthetic" *planemoides* was formed in one of the crosses. In brood 3002 (Table 5) a female *trophonissa* 2862 was mated to a male 2816 (see Part III) heterozygous for *leighi/hippocoonides (niavioides)* and which, therefore, carried part of the gene complex for the Abyssinian race *antinorii*. Neither of the butterflies had *planemoides* in their known ancestry. The brood produced eight *hippocoon(ides)*, six *trophonissa*, two normal-looking *planemoides* and two *proto-planemoides* females. In contrast when *leighi* from race *cenea* (not carrying part of the *antinorii* gene complex) is crossed with race *dardanus*, the *leighi* pattern (which is nonmimetic) does not appreciably alter, and *planemoides* is not produced (see brood 1462, Table 5). Although the complete relationship of the two forms is not yet fully worked out, it appears that with a certain combination of modifiers *leighi* can be converted to *planemoides*, and in this connection FORD (1936) has pointed out that the patterns of *planemoides* and *leighi* are rather similar. It is of interest that in brood 3002 the expected *salaami* which is the recognizable heterozygote between *leighi* and *trophonius* (see Part I) did not appear. The reason for this is not known, but it is certain that *proto-planemoides* cannot have been of this genetic constitution since if it were it would have segregated in brood 3176 (Table 5). Whether *planemoides* is an allelomorph of the other forms has not yet been established, but it seems probable that it will prove to be so.

In summary, therefore, the following are the findings in race *dardanus:*

(1) F. *trophonissa* is almost certainly dominant to f. *hippocoon.*

(2) F. *niobe* is dominant to f. *hippocoon.*

TABLE 5

Dominance relationships of f. planemoides (*race* dardanus)

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Offspring					
			Males	*planemoides* females	*proto-planemoides* females	*hippocoon* or *h'(ides)* females	*leighi* females	*trophonissa* females
1462	*leighi* race *cenea* H^Lh	race *dardanus* *hh*	11	0	0	5	*5	0
2863	*planemoides* wild	race *dardanus* wild	11	5	0	5	0	0
3002	*trophonissa* 2862 H^Th	H^Lh carrying part of gene complex of *antinorii* Brood 2816 (Part III)	20	2	2	8	0	6
3015	*planemoides* 2863	race *dardanus* *hh*	15	4	0	9	0	0
3049	*planemoides* 2863	*hh*	8	0	3	2	0	0
3176	*proto-planemoides* 3002	*hh*	4	0	3 (1 a gynandromorph)	1	0	0

* No breakdown of *leighi pattern*, c.f. brood 3002.
H^Lh = *leighi/hippocoonides.*
H^Th = *trophonissa/hippocoon.*
hh = *hippocoon.*

(3) F. *niobe* is sometimes dominant to f. *cenea* from South Africa and sometimes forms a recognizable heterozygote with it.

(4) Although f. *niobe* is usually inherited as a unit it can be synthesized by combining f. *trophonissa* with f. *planemoides*, and the frequency of the two forms makes it likely that this, in fact, may sometimes happen in the wild.

(5) F. *planemoides* is dominant to f. *hippocoon.*

(6) F. *planemoides* and f. *proto-planemoides* have been synthesized in the laboratory in a race cross. The parents were a male carrying South African *leighi* and also part of the gene complex of *antinorii* and a female *trophonissa* from race *dardanus.*

(7) The nonmimetic pattern of f. *leighi* from South Africa does not alter appreciably when this form is crossed with race *dardanus* from the west coast, where *leighi* does not occur.

(8) F. *cenea* from race *dardanus* has not so far been tested with f. *hippocoon* but in a race cross using f. *cenea* from South Africa, *cenea* was dominant to *hippocoon.* There was variable breakdown in the *cenea* mimetic pattern according to the area from which *hippocoon* was obtained.

(9) It is not yet known whether the various forms of *dardanus* are allelomorphic, but it is likely that they will prove to be so.

RACE POLYTROPHUS

This race inhabits the mountain range in the center of Tanganyika Territory and Kenya Colony on the east side of Lake Victoria. It thus occupies a position between the transitional race *meseres* and race *tibullus* (Figure 1).

It is characteristic of race *polytrophus* that some of the female forms are imperfect mimics, and these usually possess some yellow male-like pigment which can be identified by its fluorescence under ultraviolet light. The incompletely developed forms corresponding to the different mimics have the prefix *proto-* added to each.

We have not as yet investigated race *polytrophus* extensively, but we have obtained a certain amount of information regarding some of the forms. Furthermore, we have produced butterflies of the "*proto-*" form in various hybrids between the yellow nonmimetic forms (both from Abyssinia and from Madagascar) and butterflies from race *cenea* and race *dardanus*. These are discussed in Part III. The following are the forms of race *polytrophus* which we have bred:

F. proto-cenea.

This form includes all specimens of *cenea* which possess fluorescent yellow pigment. FORD, quoting VAN SOMEREN's data on the frequency of the *polytrophus* forms at Nairobi, shows that out of 150 females, 47 were normal *cenea* and 18 *proto-cenea*. The relative frequency of these is of importance when analysing the broods bred by VAN SOMEREN and also reported in FORD's monograph (1936). Thus in 19 families where the female parent was *cenea*, there were produced 91 *cenea* and 36 *proto-cenea* butterflies (28 percent of the latter form). On the other hand, where *proto-cenea* was the parent, the totals in 12 families were 31 *cenea* and 49 *proto-cenea* (61 percent of the latter form). These figures strongly suggest that *proto-cenea* is either dominant or epistatic to *cenea*. Our own data support this view because, as is shown in Table 6a, in two broods where a *proto-cenea* female was mated to a male which could not have carried *cenea* this form was produced.

VAN SOMEREN's data (given later) also suggest that *proto-cenea* (in this instance from race *tibullus*) is dominant to *hippocoonides*, and our data demonstrate this for race *polytrophus*. Thus in brood 2629 (Table 6b) a *hippocoon(ides)* female with no race *polytrophus* in its ancestry, when mated to a *polytrophus* male with seven *proto-cenea* and two *cenea* sisters, produced one *proto-cenea*, three *cenea* and five *hippocoon(ides)* females in its offspring. This brood also proves that the gene controlling *proto-cenea* is not on the Y chromosome since if it were, *proto-cenea* could not have appeared. Moreover, several broods prove that the form is not sex-linked on the X chromosome—for example, brood 2624, where the male could not have been carrying *proto-cenea*, and yet this form appeared in the offspring (Table 6b).

TABLE 6

Relationship between proto-cenea *and* cenea

a. Race crosses showing dominance of *proto-cenea* to *cenea*. In each family the father is from a race where *proto-cenea* does not usually occur.

Brood no.	Origin and form of mother	Origin of father	Offspring: Males	*proto-cenea* females	*cenea* females	*natalica* females	*hippocoon(ides)* females	*trophonius* females
2626	*proto-cenea* race *polytrophus*	*race cenea* (known not to be carrying f. *cenea*)	6	3	1	4	0	3
2801	*proto-cenea* 2629 (see b below)	race *dardanus* (known not to be carrying f. *cenea*)	42	4	13 α	0	16	0

b. Families showing (1) dominance of f. *proto-cenea* to f. *hippocoon(ides)*, (2) autosomal inheritance of f. *proto-cenea* and (3) probability that *proto-cenea* and *cenea* are not allelomorphs.

Brood no.	Form and origin of mother	Origin of father	Offspring: Males	*proto-cenea* females	*cenea* females	*hippocoon(ides)* females
2624	*proto-cenea* race *polytrophus*	race *cenea* (known not to be carrying f. *cenea*)	12	3	0 α	5
2629	*hippocoon(ides)* (no race *polytrophus* in ancestry)	race *polytrophus* male β	14	1 γ	3	5
2913	*hh* race unknown	race *polytrophus* 2748 (Table 8)	15	12	0	7

α Plus one *cenea* missing and therefore not scorable for *proto-cenea*.
β This male had two *cenea*, seven *proto-cenea* and one *poultoni* sisters.
γ This female fluoresced only slightly but was the mother of 2801 which produced *proto-cenea* in her offspring when mated to a race *dardanus* male which could not have been carrying the fluorescent form (see Table 6 a).

Allelomorphism: The intensity of fluorescence in the *proto-cenea* examined by us appeared to depend on how little ("white" *cenea*) or how much ("brown" *cenea*) nonfluorescent buff pigment was present. The former fluoresced brilliantly, the latter little or not at all. This masking of the fluorescence by the brown pigment makes the scoring of fluorescence rather unreliable, and this is particularly important when dealing with the question of allelomorphism.

The data quoted by FORD (1936) suggest that *proto-cenea* and *cenea* are not allelomorphs since on several occasions *hippocoonides* females mated to wild males have produced all three forms i.e., *proto-cenea*, *cenea*, and *hippocoonides*, whereas if they were alleles, only two of them could have appeared. Neverthe-

less, in the wild there is always the possibility that the female has been mated to several males of different genotypes. In the present work this cannot happen, but our data on allelomorphism is scanty. However, in brood 2629 (Table 6b) all three forms appeared, and in brood 2732 (Table 7) a *hippocoon(ides)* mother produced *proto-cenea*, *cenea* and *poultoni*. Although these broods suggest that *proto-cenea* and *cenea* are not allelomorphs, yet because of the scoring difficulty it may be that the results are due to modifiers affecting the amount of buff pigment and that *cenea* and *proto-cenea* are in fact allelomorphic.

F. poultoni

This is an orange butterfly not unlike *niobe*, but less rayed on the hindwing and with a grey or fawn body with black dots (Plate, No. 7). We have obtained two forms, one pale and the other bright orange, and the exact relationship between these is not yet established. However, in the vast majority of cases, the *poultoni* offspring are of the color carried or expressed by the parent, though the occasional exception occurs due possibly to the presence of modifiers (see brood 2732 and 3096, Tables 7 and 8).

Autosomal inheritance of f. poultoni: Broods 2732 and 2750 show that the gene or genes controlling both bright and pale *poultoni* are not sex-linked on the Y chromosome, and broods 2892 and 3075 show that neither is carried on the X. It is therefore clear that the genes responsible for both forms of *poultoni* are carried on an autosome (Table 7).

Dominance relationships: (a) f. pale poultoni: In brood 2750 (Table 7) a female *hippocoon(ides)* which could not have carried *poultoni*, mated to a male who

TABLE 7

Autosomal inheritance of bright and pale poultoni

			Offspring							
Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Males	pale *poultoni* females	bright *poultoni* females	*cenea* females	recognizable heterozygote *poultoni*/*cenea* females	*hippocoon(ides)* females	yellow *hippocoon(ides)* females	*dorippoides* females
2732	*hippocoon(ides)* *hh*	wild (from *poultoni* mother)	36	1	17	*16	0	0	0	0
2750	*hippocoon(ides)* *hh*	wild (from *poultoni* mother)	37	5	0	0	0	9	0	0
2892	pale *poultoni* 2750	(not carrying *poultoni*) H^ch	23	3	0	†8	6	4	0	0
3075	bright *poultoni*	H^yh	11	0	4	0	0	4	3	6

hh = *hippocoon(ides)*.
H^ch = *cenea/hippocoon(ides)*.
H^yh = yellow/*hippocoonides*. H^y is the allelomorph controlling the male-like pattern (see Part III).
* Of these 16 *cenea* females one showed strong, one slight and ten a trace of fluorescence. Four showed no fluorescence. All were "brown" *cenea*.
† Six of these *cenea* fluoresced and two were missing.

had a *poultoni* mother, (but whether of the bright or pale form is unknown) produced nine *hippocoon(ides)* and five pale *poultoni* females. This shows that pale *poultoni* is dominant to *hippocoon(ides)*. Broods 3300 and 3365 confirm this (Table 8).

(b) f. bright poultoni: Brood 2732 (Table 7) was a mating between a female *hippocoon(ides)* (which could not have carried *poultoni* or *cenea*), to a male whose mother was a wild *poultoni* of unknown color. The offspring of brood 2732 were 16 *cenea*, 17 bright *poultoni* and one pale *poultoni* female. This shows that the father must have been heterozygous *cenea/poultoni* (see below, allelomorphism). As no *hippocoon(ides)* appeared in brood 2732 it is clear that bright *poultoni* also is dominant to *hippocoonides*.

When crossed with *cenea* both bright and pale *poultoni* usually form a recognizable heterozygote with *cenea* pattern and *poultoni* coloring (H^cH^{bp} or H^cH^{pp}, called orange and buff *cenea* respectively)—as also occurs occasionally when both *niobe* and *leighi* are crossed with *cenea*. These *cenea/poultoni* heterozygotes have appeared in broods 2747, 2748 and 2892, (Table 8), and on three occasions when they have been mated to males homozygous for *hippocoon(ides)* their offspring have segregated for *poultoni* and *cenea* only, (broods 3054, 3055 and 3096, Table 9).

The only evidence which we have regarding *poultoni* and *trophonius* suggests that they, too, form a recognizable heterozygote. Thus, in brood 2927 (Table 9) a hybrid female heterozygous for *trophonius* and the yellow nonmimetic form *antinorii* (from brood 2741, see Part III) when mated to an unknown male, produced one male and one female of *trophonius* pattern and color, but with the white subapical spot replaced by pale orange. Brood 3080 (Table 9) was a mating between this female and a male homozygous for *hippocoon(ides)*, and from it only *trophonius* (13) and pale *poultoni* (15) females were obtained—no other form appearing. Clearly, therefore, the unknown male carried pale *poultoni*, and the mother of brood 3080 was a heterozygote *trophonius*/pale *poultoni*.

The dominance relationships of bright and pale *poultoni* to f. *leighi*, f. *natalica*, f. *planemoides* and f. *niobe* are not yet known.

Allelomorphism (Table 9): In brood 3054 a female pale *poultoni/cenea* heterozygote 2892 (which can only have received *poultoni* from her mother and *cenea* from her father) mated to a male homozygous for *hippocoon(ides)* produced four *cenea* females and one bright *poultoni* female. Brood 3055 was a similar mating (a female 2892 pale *poultoni/cenea* with an *hh* male) and this produced eight *cenea* and three pale *poultoni* females. Brood 3096 again was between a female pale *poultoni/cenea* heterozygote 2892 and a male homozygous for *hippocoon(ides)*. This produced one male and one female bright *poultoni*. Broods 3054, 3055 and 3096, therefore, show that *poultoni* and *cenea* are allelomorphs since no *hippocoon(ides)* females appeared.

Brood 3080 shows that pale *poultoni* and *trophonius* are allelomorphs. In this mating a female 2927, heterozygous for *trophonius* and pale *poultoni* (each form being inherited from a different parent), when mated to a male homozy-

TABLE 8

Dominance relationship of bright and pale poultoni *to* hippocoonides *and* cenea

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Offspring: Males	bright *poultoni* females	pale *poultoni* females	*hippocoonides* females	recognizable heterozygote *cenea*/*poultoni* females	*cenea* females
2747	race *polytrophus* f. *cenea* H^ch	race *polytrophus* from wild *poultoni* mother	4	0	1	0	3	3 α
2748	race *polytrophus* f. *proto-cenea* H^ch	race *polytrophus* from wild *poultoni* mother	9	0	0	0	5	9 β
2892	race *polytrophus* pale *poultoni* 2750 $H^{pp}h$ (see Table 7)	race *polytrophus* H^ch	23	0	3	4	6	8 γ
3096	recognizable heterozygote *cenea*/bright *poultoni* δ 2892	*hh*	1	1	0	0	0	0
3300	pale *poultoni* 3055 $H^{pp}h$ (see Table 9)	*hh*	2	0	2	2	0	0
3365	pale *poultoni* 3080 $H^{pp}h$ (see Table 9)	*hh*	7	0	8	3	0	0

H^ch = heterozygous *cenea/hippocoonides*.
hh = *hippocoonides*.
$H^{pp}h$ = pale *poultoni/hippocoonides*.
α Two *proto-cenea* and one butterfly missing.
β Five *proto-cenea*, three *cenea* plus one *cenea* missing and not scored for *proto-cenea*.
γ Six *proto-cenea* and two butterflies missing.
δ From a pale *poultoni* mother.

gous for *hippocoon(ides)* produced 13 *trophonius* and 15 pale *poultoni* females. As *poultoni* has been proved to be an allelomorph of f. *trophonius* and f. *cenea*, it follows that it is also an allele of f. *leighi* and f. *natalica*. It is also an allelomorph of the yellow nonmimetic forms of races *antinorii* and *meriones* (see Part III).

F. dorippoides

There is a form, *proto-salaami*, occurring both in race *polytrophus* and occasionally in race *meseres*, which is similar to the South African *salaami* except that the black bar between the subapical orange spot and the lower part of the forewing is less distinct. *Proto-salaami* intergrades on the one hand to f. *poultoni* where the black bar is complete and on the other to *dorippoides* where it is

TABLE 9

Allelomorphism of poultoni, trophonius *and* cenea

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Offspring					
			Males	bright *poultoni* females	pale *poultoni* females	*trophonius* females	recognizable heterozygote *trophonius*/pale *poultoni* females	*cenea* females
2927	*trophonius/antinorii* F_1 hybrid*	$H^{pp}h$	1	0	0	0	1	0
3054	recognizable heterozygote *cenea/poultoni* 2892 (see Tables 7 and 8)	*hh*	15	1	0	0	0	4
3055	recognizable heterozygote *cenea/poultoni* 2892 (see Tables 7 and 8)	*hh*	10	0	3	0	0	8
3080	recognizable heterozygote *trophonius*/pale *poultoni* 2927 (see above)	*hh*	23	0	15	13	0	0
3096	recognizable heterozygote *cenea/poultoni* 2892 (see Tables 7 and 8)	*hh*	1	1	0	0	0	0

* From brood 2741, see Part III.
hh = *hippocoonides*.
$H^{pp}h$ = Pale *poultoni/hippocoonides*.

absent, and here the forewing, except for the black border, is entirely orange, (Plate, No. 8). *Dorippoides* received its name because it resembles the model *Danais chrysippus dorippus*, but FORD (1936) doubts whether it is in fact a mimic.

F. *dorippoides* appeared in three of our race crosses though in none of them had it occurred in the known ancestry of either parent. In each instance, however, the butterflies inherited the gene controlling either bright or pale *poultoni* from one parent and some part of the *antinorii* gene complex from the other, and this combination can certainly produce *dorippoides*. The shade of orange of the *dorippoides* corresponds to that of the bright or pale *poultoni* parent. The matter is dealt with in more detail in Part III. Our synthesis of *dorippoides* agrees with the hypothesis which we have also put forward below to explain VAN SOMEREN's data for the same form, referred to under *proto-salaami*.

F. proto-trophonius (lamborni)

This is a *trophonius*-like insect but with the subapical spot and part of the orange area of the forewing pale yellow (FORD 1936. Plate 2). It has not appeared in our wild stock but we have on one occasion produced a butterfly resembling it by crossing f. *trophonius* from South Africa with the nonmimetic race *meriones* from Madagascar, (discussed in Part III). Though this might suggest that *proto-trophonius* is a modified *trophonius*, yet it seems highly probable that the two forms are in fact controlled by different genes because (1) on backcrossing a *trophonius* from a South African × *polytrophus* hybrid to *polytrophus* for four generations we did not produce *proto-trophonius*, the orange insect remaining similar to the original *trophonius*, despite the fact that *proto-trophonius* is 17 times commoner than *trophonius* in race *polytrophus* (FORD 1936). (2) f. *trophonius* × f. *planemoides* give f. *niobe*, whereas *proto-trophonius* × f. *planemoides* probably give *proto-salaami*, a less extreme form of f. *dorippoides* (see VAN SOMEREN's data).

In summary, therefore, the following are the findings in race *polytrophus:*

(1) *Proto-cenea.* This is dominant to *hippocoon(ides)* and either dominant or epistatic to *cenea.* The data suggest that *proto-cenea* and *cenea* are *not* allelomorphs, but difficulties in scoring the two forms make this conclusion very tentative.

(2) Both bright and pale *poultoni* are dominant to *hippocoon(ides).*

(3) Both bright and pale *poultoni* usually form a recognizable heterozygote with *cenea*, the insects having the *cenea* pattern and the *poultoni* coloring.

(4) The evidence (from one insect only) suggests that *poultoni* and *trophonius* also form a recognizable heterozygote.

(5) F. *poultoni*, f. *cenea* and f. *trophonius* are all allelomorphs, and therefore the gene for *poultoni* is at the same locus as all the other South African forms. (It is also an allelomorph of the yellow nonmimetic forms of races *antinorii* and *meriones*, see Part III).

(6) We have synthesized f. *dorippoides* from parents in whose known ancestry the form has not appeared.

(7) A form resembling f. *proto-trophonius (lamborni)* has been produced by crossing f. *trophonius* with f. *meriones* from Madagascar, but it has been shown that this form in *polytrophus* is probably controlled by a different gene from that of f. *trophonius.*

RACE MESERES AND RACE TIBULLUS (VAN SOMEREN'S DATA)

Race *meseres* (also known as the transitional race) is found in Uganda and Tanganyika Territory east of Lake Victoria. Further east it merges with race *polytrophus* and to the west with *dardanus.* It comprises seven forms of female (FORD 1936). Rarely the mimetic pattern is imperfect and when this occurs the prefix *proto-* has been added as in *polytrophus.* Race *tibullus* is found for an uncertain distance north of the Mombasa district and extends southwards to Delagoa Bay. The female forms are similar to those in race *cenea*, (see Part I)

but in the northern part of its range where it merges into race *polytrophus* imperfect mimics are occasionally found.

We have no personal experience of either *meseres* or *tibullus*, and we are indebted to DR. VAN SOMEREN who has allowed us to quote his breeding experiments with these two races.

It will be seen from his family S1 in our Table 10 that a female *hippocoonides* from race *tibullus* mated to a wild *tibullus* male produced nine *proto-cenea* and eight *hippocoonides* females. Four of these females (one *proto-cenea* and three *hippocoonides*) were used in race crosses with *meseres* males. It will be seen that the results strongly suggest, but do not prove, that *proto-cenea* is dominant to *hippocoonides* as the chance of the *meseres* male parent in S3 being heterozygous for *proto-cenea* is small, the frequency of the form being very low in this race. Because brood S1 contained no female forms other than *proto-cenea* and *hippocoonides* (nor did the F_2, F_3, or F_4 generations from this family—number

TABLE 10

VAN SOMEREN'S data

Brood no.	Brood no. and form of female	Origin of male	Offspring						
			Males	*hippo-coonides* females	*plane-moides* females	*leighi* females	*proto-trophonius* females	*proto-salaami* females	*proto-cenea* females
S1	h *tibullus*	w *tibullus*	14	8	0	0	0	0	9
S2	h (S1) *tibullus*	w *meseres*	6	2	0	1	0	0	0
S3	p-c (S1) *tibullus*	w *meseres*	8	1	0	3	0	0	5
S4	h (S1) *tibullus*	w *meseres*	20	4	6	0	0	0	0
S5	h (S1) *meseres*	w *meseres*	20	11	3	0	0	0	0
S6	h *meseres*	w *meseres*	21	12	0	0	0	0	0
S7	p-p *meseres*	w *meseres*	6	0	0	0	0	3	0
S8	h (S6) *meseres*	(S7) *meseres*	29	0	9	0	10	0	0
S9	p (S8) *meseres*	(S8) *meseres*	17	6	8	0	0	8	0
S10	p-t (S8) *meseres*	(S8) *meseres*	6	0	3	0	5	0	0
S11	p-t (S10) *meseres*	(S10) *meseres*	10	1	12	0	2	0	0
S12	h (S9) *meseres*	(S9) *meseres*	3	0	3	0	0	0	0

* Brood S1 is pure race *tibullus* and four subsequent broods from it (not shown in table) have produced nothing but *proto-cenea* and *hippocoonides* (numbers not given). The males of all the other broods are of race *meseres* as are the females of broods S6 to S12.

h=*hippocoonides*. p=*planemoides*. l=*leighi*. p-t=*proto-trophonius*. p-c=*proto-cenea*. p-p=*proto-planemoides*. p-s=*proto-salaami*. w=wild.

not given by VAN SOMEREN and not shown in Table 10), it is highly probable that the brood was not carrying the genes for *planemoides* or *leighi*. His data (broods S2, S3, S4 and S5), therefore, agree with ours in showing *leighi* dominant to *hippocoonides* and in addition to this they show that f. *planemoides*, as it is not sex-linked, is also dominant to *hippocoonides* in these race crosses. In Table 10 it will be found also that within the race *meseres*, a female *proto-planemoides*, an insect which looks intermediate between f. *planemoides* and f. *leighi* (Plate, No. 6), mated to a male of unknown constitution produced three *proto-salaami* females and six males (brood S7). One of these S7 males mated to a *hippocoonides* produced only *proto-trophonius (lamborni)* and *planemoides* (brood S8). Thus, as *proto-trophonius* is not sex-linked (FORD 1936), it is also dominant to *hippocoonides*, a view fully in agreement with the other broods in the same table. If the genes controlling *planemoides* and *proto-trophonius* are not allelomorphs (though they probably are, see below), then *proto-trophonius* must be dominant; if it were not it would have appeared among the offspring of brood S9. In other words, the parents of S9 must have both been heterozygous for *planemoides* and neither can have carried *proto-trophonius* since none appeared in S9. On the assumption that the two are controlled by independent loci we cannot assume that in this brood *proto-salaami* is a combination of *planemoides* and *proto-trophonius* since no *proto-salaami* appeared in S8.

VAN SOMEREN's data give useful information about the inheritance of *proto-salaami*. An F_2 generation (brood S9) produced *proto-salaami*, a form which although present in the female sibs of one male grandparent was not present among the sibs of the parents. This suggests at first sight that *proto-salaami* is dominant to *hippocoonides*, as is strongly indicated in FORD's paper, but cannot express itself in the presence of *planemoides* or *proto-trophonius*. However, brood S9 is inconsistent with this view which would necessitate a ratio of 12:3:1 or a 12:2:2 *planemoides* to *proto-salaami* to *hippocoonides*. Moreover, the families in FORD's monograph strongly suggest that *proto-salaami* is also dominant to *proto-trophonius*, which is not in accord with the newer data. A less unlikely hypothesis is that in VAN SOMEREN's families *proto-salaami* is a recessive, and that brood S9 is an example of a 9:4:3 ratio. The other broods do not contradict this hypothesis, if we assume that the male parent of S8 was heterozygous for the gene producing *proto-salaami*, and that brood S10 resulted from two parents neither of which was carrying the gene. Whatever view we take, it is clear that *proto-salaami* is genetically controlled in two distinct ways. Firstly, from FORD's paper (1936) and our own results with f. *poultoni* (a form of *proto-salaami*) it can be seen that *proto-salaami* can be inherited as a single unit dominant in effect; secondly, from VAN SOMEREN's results it is clear that *proto-salaami* is either a recessive or, more likely, the heterozygote between allelomorphs controlling *planemoides* and *proto-trophonius*. It has often been pointed out that the pattern of *planemoides* is easily derived from that of *leighi*, and in view of the results with f. *salaami* from South Africa it seems more probable that *planemoides* and *proto-trophonius* are allelomorphs at the same locus as those

controlling all the South African forms, and that *proto-salaami* is the recognizable heterozygote between *planemoides* and *proto-trophonius*. Brood S8 is fully consistent with this view if the male parent (and his three female sibs, brood S7) were a heterozygote. On this hypothesis there is, however, a deficiency of *proto-trophonius* in brood S9 and of *proto-salaami* in brood S10 and S11. Nevertheless, because there seems to be some deficiency of *hippocoonides* in broods S10 and S11 combined and particularly of *proto-trophonius* in brood S11 (even if we assume that the forms are controlled by independent loci), it may be that there was abnormal segregation due to viability effects. This is a likely proposition as VAN SOMEREN reported that the butterflies in brood S11 were particularly undersized, and it is here that there is the notable excess of *planemoides* and deficiency of *proto-trophonius*.

In summary, therefore, VAN SOMEREN's findings are: (1) f. *proto-cenea* is almost certainly dominant to f. *hippocoonides*. (2) f. *proto-planemoides* and f. *proto-trophonius* are dominant to *hippocoonides*. (3) f. *proto-salaami* must be inherited in at least two ways, sometimes as a unit dominant to *hippocoonides* or sometimes not as a dominant but in all probability as the heterozygote between *planemoides* and *proto-trophonius*.

SUMMARY

A genetic investigation into races *dardanus*, *polytrophus*, *meseres* and *tibullus* of *Papilio dardanus*, together with some hybridization experiments between them, has been carried out.

(1) In race *dardanus* it was found that f. *trophonius*, f. *niobe* and f. *planemoides* were all dominant to f. *hippocoon*, and that *trophonius* and *planemoides* gave a recognizable heterozygote resembling *niobe*. The *cenea/niobe* heterozygote was variable and not always distinguishable from *niobe*.

In race *polytrophus*, f. *proto-cenea* was found to be dominant to f. *hippocoonides* as were the two forms bright and pale *poultoni*. The last two, however, produced intermediate forms with *cenea* and are *allelomorphic* with this and all the other South African forms. Furthermore, in race *meseres*, a *poultoni*-like form (*proto-salaami*), is sometimes inherited not as a single dominant but probably as the heterozygote between *planemoides* and *proto-trophonius*.

(2) Race crosses show that when the gene complexes are disturbed by hybridization the effects of the genes become far less constant. For example, the mimetic pattern of *cenea* breaks down when the form is hybridized with the West African material where the mimicry does not occur.

(3) Dominance is commoner and more complete between forms that are sympatric as compared with those that are allopatric. This suggests that dominance has been evolved.

ACKNOWLEDGMENTS

We are very grateful to DR. E. B. FORD, F.R.S., for reading the paper and for his helpful comments.

We are also extremely grateful to DR. V. G. L. VAN SOMEREN for sending us living material of race *polytrophus* and for giving us much detailed information about his field work on *dardanus*.

We are also greatly indebted to MR. J. A. BURGESS who has been most successful in sending us living butterflies from race *dardanus* in Uganda.

Our thanks are due to the Nuffield Foundation for its generous support without which much of the work could not have been carried out.

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THE GENETICAL ANALYSIS OF CHARACTERS WITH CONTINUOUS VARIABILITY ON A MENDELIAN BASIS. II. MONOHYBRID SEGREGATION AND LINKAGE ANALYSIS

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Received September 29, 1959

THE segregation of continuous F_2 distributions has been demonstrated on the character cotyledon size in Part I "Monohybrid segregation" in WEBER (1959). The same methods were applied to the character primary leaf size for the parents and the F_1, the single measurements length (insertion to tip) $= x_4$ and area $= x_5$ being used. Figure 1 shows the average sizes of hypocotyl, cotyledon and primary leaf of the tomato varieties Professor Rudloff (=R) and Bonner Best (=B).

FIGURE 1.—The average sizes of hypocotyl, cotyledon and primary leaf of the parents R (left) and B (right).

The following single measurements are being considered:

x_1 = length of hypocotyl
x_2 = length/width ratio of cotyledon
x_3 = area of cotyledon
x_4 = length of primary leaf
x_5 = area of primary leaf

Now the discriminant function worked out in the first paper (WEBER 1959) is to be applied to an F_2 with 589 individuals and to a backcross generation with 636 individuals.

The F_1 being phenotypically uniform with parent B in the characters concerned, the discriminant functions were calculated from the collectives R and B.

The coefficients b_p for the discriminant function $X_C = f(x_2) + f(x_3)$ for the character cotyledon size are obtained from the following equation:

$$+ 2446.5333\, b_2 + 344.6667\, b_3 = + 7.6667$$
$$+ 866.5333\, b_3 = + 5.9333$$
$$b_2 = + 0.002300$$
$$b_3 = + 0.005925$$
$$\text{or } X_C = + 1\, x_2 + 2.575\, x_3$$

$F = 110$ $(n_1 = 2, n_2 = 117)$; $\frac{1}{2}$ P = 0.12 (misclassification)

The discriminant function $X_P = f(x_4) + f(x_5)$ for the character primary leaf size is obtained from the equation

$$+ 2566.7667\ b_4 + 4999.2333\ b_5 = + 20.0334$$
$$+ 14350.8667\ b_5 = + 20.7666$$

$$b_4 = + 0.015501$$
$$b_5 = - 0.003956$$
$$\text{or } X_P = + 1\ b_4 - 0.2552\ b_5$$

$F = 400$ $(n_1 = 2, n_2 = 117)$; $\frac{1}{2}$ P = 0.005 (misclassification)

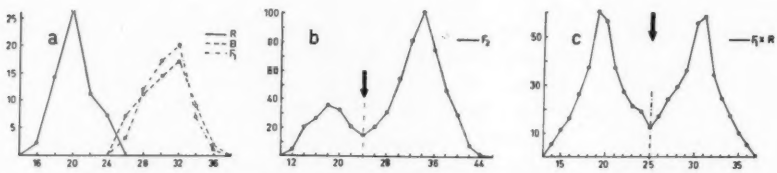


FIGURE 2.—a. Frequency distributions of the single measurement x_1 (hypocotyl length) for the parents R and B and for F_1. b. Frequency distribution of the single measurement x_1 for F_2; 3:1 segregation. c. Frequency distribution of the single measurement x_1 for the backcross generation $F_1 \times R$; 1:1 (1:0.98) segregation.

In Figure 2, the frequency distributions of hypocotyl length $(= x_1)$ for the parents, F_1, F_2, and the backcross generation have been included. The F_2 distribution (Figure 2b) clearly indicates a 3:1 segregation, and that of the backcross generation a 1:1 segregation (Figure 2c).

The frequency distributions of the single measurement x_2 for the parents R and B and for F_1 are shown by Figure 3a, for F_2 by Figure 3d, and for the backcross generation by Figure 3g. The corresponding distributions of the single measurement x_3 are shown in Figures 3b, 3e and 3h. The F_2 distributions (Figures 3d and 3e) show no segregation; nor do the distributions of the backcross generation (Figures 3g and 3h) clearly present the expected 1:1 segregation. But the distributions of the total measurements X_G for the F_2 and the backcross generation in Figures 3f and 3i reveal clear 3:1 and 1:1 segregations respectively.

The same applies to Figures 4a–4i showing the distributions of the single measurements x_4, x_5 and the total measurement X_P.

From these it may be concluded that there is monohybrid segregation in the character primary leaf size as well as in cotyledon size.

LINKAGE ANALYSIS

Investigations have revealed absolute linkage of the shorter hypocotyl to the smaller cotyledon and to the smaller primary leaf of the tomato variety R. Similarly, in variety B the longer hypocotyl is linked to a larger cotyledon

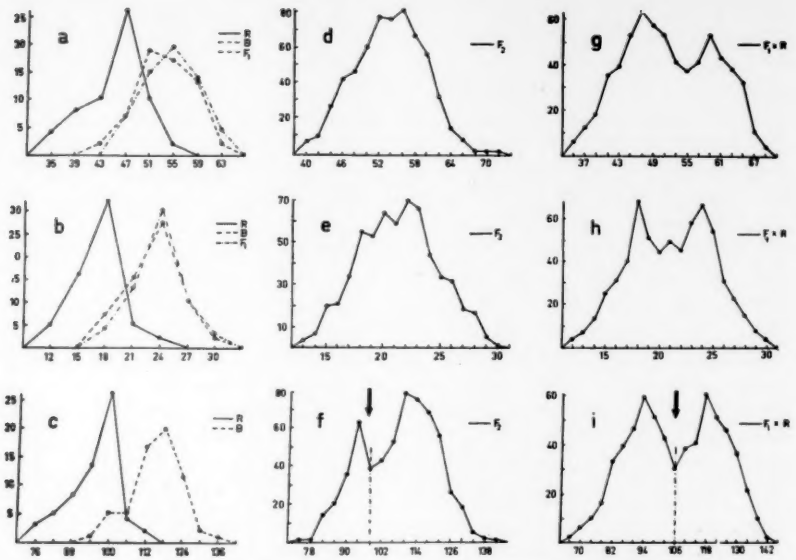


FIGURE 3.—a. Frequency distributions of the single measurement x_2 (length/width ratio of the cotyledon) for the parents R and B and for F_1. b. Frequency distributions of the single measurement x_3 (area of cotyledon) for the parents R and B and for F_1. c. Frequency distributions of the total measurement X_C of x_2 and x_3 for the parents R and B. d. Frequency distribution of the single measurement x_2 for F_2. e. Frequency distribution of the single measurement x_3 for F_2. f. Frequency distribution of the total measurement X_C of x_2 and x_3 for F_2; 3:1 (3:1.04) segregation. g. Frequency distribution of the single measurement x_2 for the backcross generation $F_1 \times R$. h. Frequency distribution of the single measurement x_3 for the backcross generation $F_1 \times R$. i. Frequency distribution of the total measurement X_C of x_2 and x_3 for the backcross generation $F_1 \times R$; 1:1 (1:0.99) segregation.

and to a larger primary leaf (see Figure 1). As only in one of the many backcrosses a total exchange of 1.8 percent was obtained, various data have been exchanged in order to demonstrate linkage analysis. Thus, this analysis is to be considered as a model (KAPPERT 1953).

The extensive table of backcross data not included in the text contains the following columns:

1) No. of individual ($= N_i$)
2) hypocotyl length ($= x_1$) (Figure 2c)
3) length/width ratio of cotyledon ($= x_2$) (Figure 3g)
4) area of cotyledon ($= x_3$) (Figure 3h)
5) total measurement ($= X_C$) of x_2 and x_3 (Figure 3i)
6) length of primary leaf ($= x_4$) (Figure 4g)

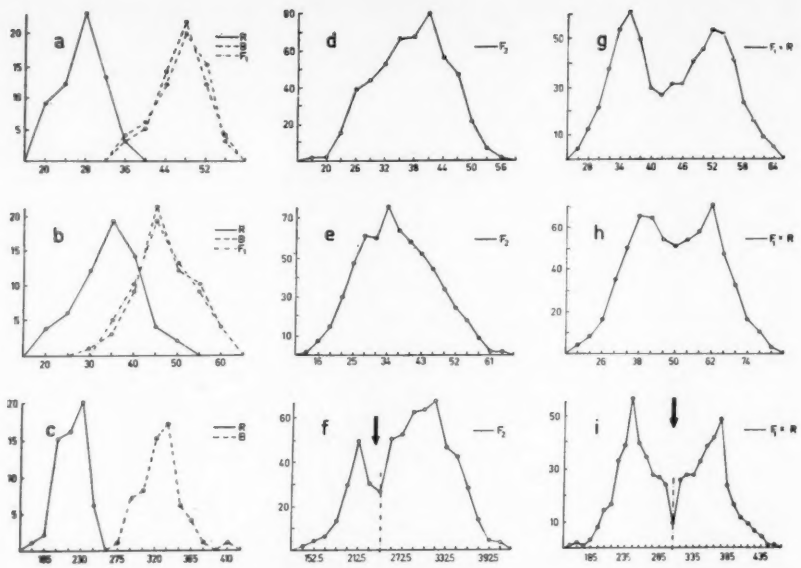


FIGURE 4.—a. Frequency distributions of the single measurement x_4 (length of primary leaf) for the parents R and B and for F_1. b. Frequency distributions of the single measurement x_5 (area of primary leaf) for the parents R and B and for F_1. c. Frequency distributions of the total measurement X_P of x_4 and x_5 for the parents R and B. d. Frequency distribution of the single measurement x_4 for F_2. e. Frequency distribution of the single measurement x_5 for F_2. f. Frequency distribution of the total measurement X_P of x_4 and x_5 for F_2; 3:1 (3:0.99) segregation. g. Frequency distribution of the single measurement x_4 for the backcross generation $F_1 \times R$. h. Frequency distribution of the single measurement x_5 for the backcross generation $F_1 \times R$. i. Frequency distribution of the total measurement X_P of x_4 and x_5 for the backcross generation $F_1 \times R$; 1:1 (1:0.97) segregation.

7) area of primary leaf ($= x_5$) (Figure 4h)
8) total measurement ($= X_P$) of x_4 and x_5 (Figure 4i)

(This table may be obtained on application to the author.)

In addition, the test data of the hypocotyl and the total measurements of the cotyledon and primary leaf are marked according to their collectives. Allocation to one of the phenotypic classes R or B in this case cannot be done simply by way of the respective critical constant (WEBER 1957), otherwise a mistaken impression of crossing over may be given. According to the extent of the misclassification, the segregation by discriminant function is incomplete. An overlapping zone remains, i.e., a zone in which a character might belong to either collective by reason of its measurement. Individuals of which one, two or all three measurements (x_1, X_C, X_P) of the linkage group fall into this zone must therefore be excluded.

With the relatively large number of individuals ($N = 636$) in the distributions x_1, X_C and X_P (Figures 2c, 3i, 4i) there are two distributions each; these deviate, however, on both sides of the notch (arrow) from the normal distribution by addition of two distributions in the overlapping zone. This means that the borders of this zone are at the point where the distributions begin to deviate from the normal distribution.

It is best to fix these borders by way of a probit transformation. As a clear 1:1 segregation is indicated by the distributions in Figures 2c, 3i and 4i, we let $\frac{1}{2} N = 318 = 100$ percent. The sum frequency graph is set up from the left in the left-hand distribution and *vice versa*. Table 1 illustrates this process for the distribution of X_C (Figure 3i).

Figures 5, 6, and 7 show the sum frequency graphs transformed into straight lines. The points deviating from the probit straight line at the same time represent the deviation from the normal distribution. They are indicated by arrows; the zone between these points is the overlapping zone. The measurements x_1 24–26 comprising 48 individuals, for the character hypocotyl; the total measurements X_C 1000–1119 comprising 110 individuals, for the character cotyledon; and the total measurements X_P 290–329 comprising 83 individuals, for the character primary leaf—all fall into the overlapping zone.

TABLE 1

Table of frequency distribution for the total measurement X_C*

Class	$F_1 \times R$	Sum		Sum percent	
60.0 – 63.9	0	0		0.0	
64.0 – 67.9	2	2		0.6	
68.0 – 71.9	6	8		2.5	
72.0 – 75.9	10	18		5.7	
76.0 – 79.9	16	34		10.7	
80.0 – 83.9	33	67		21.1	
84.0 – 87.9	39	106		33.4	
88.0 – 91.9	46	152		47.8	
92.0 – 95.9	59	211		66.2	
96.0 – 99.9	51	262		82.0	
100.0 – 103.9	42	304		95.8	
104.0 – 107.9	30	318	318	100.0	100.0
108.0 – 111.9	38		302		95.0
112.0 – 115.9	40		264		83.0
116.0 – 119.9	60		224		70.6
120.0 – 123.9	51		164		51.7
124.0 – 127.9	46		113		35.6
128.0 – 131.9	36		67		21.0
132.0 – 135.9	21		31		9.7
136.0 – 139.9	8		10		3.1
140.0 – 143.9	2		2		0.6
144.0 – 147.9	0		0		0.0

* Further explanation in the text

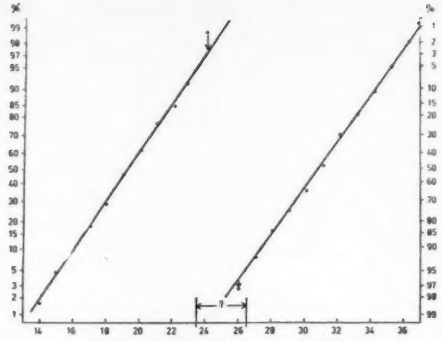


FIGURE 5.—Sum frequency of hypocotyl length x_1 in Figure 2c. (Further explanation in the text.)

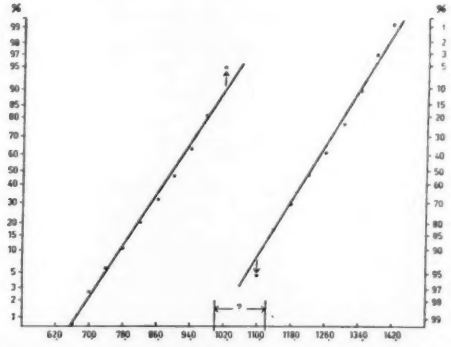


FIGURE 6.—Sum frequency of the total measurement X_C (cotyledon) in Figure 3i. (Further explanation in the text.)

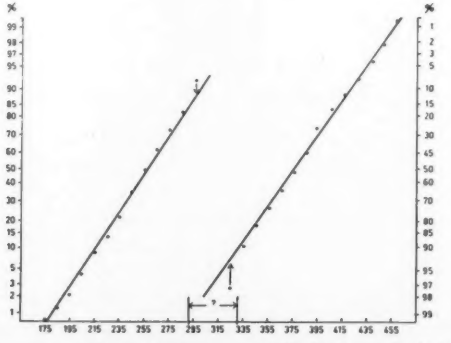


FIGURE 7.—Sum frequency of the total measurement X_P (primary leaf) in Figure 4i. (Further explanation in the text.)

These individuals are marked with a question mark (?) in the above mentioned table (not presented in the present paper). Altogether, 165 such individuals have one, two or three question marks each. Thus, on subtracting these 165 plants there remain 471 plants for linkage analysis.

Let the factor for the hypocotyl be *H*— or *hh* respectively, for the cotyledon *C*— or *cc* and for the primary leaf *P*— or *pp*; we get the following pattern:

Linkage of three genes, H, C, P *and their alleles*

B × R	*HHCCPP* × *hhccpp*
F_1	*HhCcPp*
F_1 × R	*HhCcPp* × *hhccpp*

In free segregation one would expect equal shares of the genotypes:

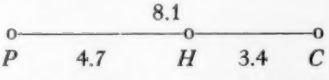
F_1 / R	*HCP*	*HCp*	*HcP*	*Hcp*	*hCP*	*hCp*	*hcP*	*hcp*
hcp	*HCP*	*HCp*	*HcP*	*Hcp*	*hCP*	*hCp*	*hcP*	*hcp*
	hcp	*hcp*	*hcp*	*hcp*	*hcp*	*hcp*	*hcp*	*hcp*

In the model, the following figures resulted:

HCP	*HCp*	*HcP*	*Hcp*	*hCP*	*hCp*	*hcP*	*hcp*
218	13	11	0	0	5	9	215

Total exchange = $(13 + 11 + 5 + 9) / 471 = 8.1$ percent
Single exchange $H/pp = (13 + 9) / 471 = 4.7$ percent
Single exchange $H/cc = (11 + 5) / 471 = 3.4$ percent

Localization of these genes (separating units) would be represented as follows:



This model shows that it is possible to carry out linkage analysis combined with gene localization for characters with continuous variability.

It should, however, be pointed out that by excluding individuals from the overlapping zone, a deviation from the 1:1 ratio has occurred in the segregation ratio of the character hypocotyl length. Originally, H—:$hh = 1{:}0.98$; after exclusion, H—:$hh = 1{:}0.95$ ($\chi^2 = 0.36$; $P = 0.56$). The usefulness of the method described in this paper is not likely to be limited by such deviations, provided they do not become excessive through the above mentioned exclusion.

SUMMARY

The method described in WEBER (1959) for genetical analysis of characters with continuous variability on a Mendelian basis by means of a discriminant

function, is applied to an F_2 generation with regard to the inheritance of the characters cotyledon size and primary leaf size in two varieties of tomato. Linkage analysis of characters with continuous variability is illustrated by a model.

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TWO-WAY SELECTION FOR BODY SIZE IN RATS, WITH OBSERVATIONS ON SIMULTANEOUS CHANGES IN COAT COLOR PATTERN AND HOOD SIZE[1]

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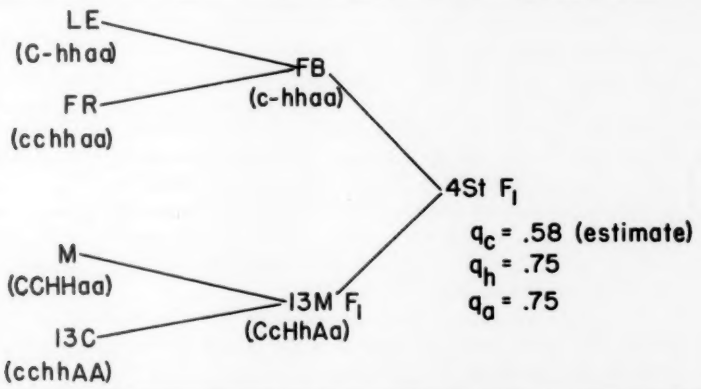
Received September 30, 1959

IN the course of a two-way selection experiment for body size in rats, we observed a number of differences, other than size, between the resulting lines (T. F. ZUCKER 1953, 1957). The question arose as to whether the observed correlations with body size were chance or obligate. To answer this question we are carrying on a second size selection experiment quite independent of the first. Results for the various characters possibly correlated with size and observed in both experiments will be reported elsewhere. Here we wish to describe the second size selection experiment now in its tenth generation of selection and will consider the effects of body size selection and restricted population size upon some characters not observed in the first experiment—coat color and pattern as determined at three major loci and the many loci affecting size of hood.

TABLE 1

*Constitution of foundation stock**



* Six or seven rats of each stock were used, about half of each sex, not sibs or half-sibs.

[1] This investigation was supported by research grants C-2471 and C-4241 from the National Institutes of Health.

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MATERIAL AND METHODS

Foundation stock

The 4St stock was set up (see Table 1) from four established rat stocks each of which has been maintained for many years as a closed colony with avoidance of close inbreeding. LE (Long-Evans) was obtained from the branch colony established in the 1920's in the Anatomy Department of Columbia University by DR. P. E. SMITH. The Long-Evans stock originated somewhat earlier in California, from a crossing of laboratory albino females with local wild males. M (Merck) was developed at the Merck Institute for Therapeutic Research, Rahway, N. J., also from a cross between laboratory albino females (Sherman stock) and local wild males. M is a true-breeding black self extracted from this cross some years ago. Branches of this black stock are located at the National Institutes of Health (there called NIH black) and at Columbia University (formerly colony of DR. MAURICE SHILS, Public Health Institute, now of DR. W. B. STEWART, Department of Pathology). FR (Food Research) is originally of Wistar origin (Wistar noninbred albino colony brought to the Institute by DR. H. H. DONALDSON) and obtained from the branch colony of the Food Research Laboratories, Long Island City, N. Y., DR. B. L. OSER. 13C is of originally Sherman stock (colony of DR. H. C. SHERMAN, Department of Chemistry, Columbia University), which was in turn a branch of the Osborne-Mendel stock of the Connecticut Agricultural Experiment Station. This was originally a rather small rat. At Connecticut it has been bred up to a very large size, and in our hands it was selectively bred both up and down for body size. 13C was a moderately large line, but not the largest obtained from this first size selection experiment. After ten generations of selection, selection was relaxed for another eight generations. Of the other stocks employed, FR is definitely small, M and LE are middle-sized as rat stocks go.

The foundation stock 4St was heterozygous at three loci affecting coat color or pattern—albinism (*c*,*C*), hood-self (*h*,*H*) and agouti (*a*,*A*). *C* (nonalbino) and *A* (agouti) are fully dominant; *H* (self) is not. The initial allelic frequencies for these three genes, shown in Table 1, were calculated from the constitutions of the parent stocks which were either apparent from their phenotypes or established by the results of the crosses. Thus FR is phenotypically albino (*cc*). LE is heterozygous at the three loci in question, but we chose to use only phenotypically black (i.e., nonagouti) hooded individuals, which are *hhaa*, with one *C* and the other allele at this locus either *C* or *c*. The offspring of the FR × LE cross were, by litters, either all phenotypically black hoods, or about half black hoods, half albinos. This confirms the *hhaa* constitution for the FR rats, which we had expected since it is the known constitution of the Wistar noninbred albino colony. It also fixes the condition at the albino locus for each LE rat used.

13C is albino (*cc*). M is a true breeding black self, therefore *CCHHaa*. The cross turned out all agouti, with variable white patches on the belly and at the wrists. Heterozygotes at the hooded self locus are known to show such white spotting as appeared here; this is the pseudo-Irish, occasionally confused in the past with a true-breeding Irish which is the homozygote of another allele h^i at the

hooded locus (MUDGE 1908; CASTLE 1940, Figures 62 and 74 for *hH* Irish, Table 40 for h^1; see also CASTLE 1951). The 13C is, therefore, indicated to be *AA* and *hh*. Further results of this cross are summarized in Table 2 covering F_2 and backcrosses. No other known coat color variants have appeared in the two lines selected for large and small body size, called 4StL and 4StS, nor in the 13M stock resulting from the 13C × M cross, which has been carried on for nine generations. The original M stock occasionally shows a white spot in the groin indicating some spotting factor, but none of the individuals used showed this, and it has not turned up since.

Breeding plan

The F_1 generation of 4St was subjected to some size selection but not according to the same formal scheme as all the later generations. The F_1 (parents 13M and FB) comprised 14 litters, and one of each sex was chosen from each litter at random. They were later mated according to size, and the six largest pairs were called 4StL; the six smallest were called 4StS. This was then a mass selection procedure of low selectivity (nearly 50 percent retained). Beginning with the second 4St generation, selection was handled according to the scheme used by FALCONER (1953a) in his mouse selection studies with selection purely within litters. In each generation there are six litters, each of four females and four males, totalling 48 rats. The best (i.e., largest or smallest as the case may be) male and best female of each litter are the breeders for the next generation, so that 25 percent of the population is selected, and there are six breeding pairs per generation. The most distantly related animals are mated, and if the female of litter A is mated with the male of litter B, the reverse pairing is avoided. We have

TABLE 2

Cross between 13C (albino) and M (black self)

		Albino‡ *cc*	Colored *C-*	Hooded black *C-hhaa*	Irish black *C-hHaa*	Self black *C-HHaa*	Hooded grey *C-hhA-*	Irish grey *C-hHA-*	Self grey *C-HHA-*
F_1									
	Found	0	88	0	0	0	0	55	0
	Expected†	0	88	0	0	0	0	55	0
F_2*									
	Found	66	264	11	29	21	28	78	32
	Expected	82.5	247.5	12.4	24.9	12.4	37.3	74.6	37.3
$F_1 \times M$									
	Found	0	74	0	15	12	0	17	19
	Expected	0	74	0	15.8	15.8	0	15.8	15.8
$F_1 \times 13C$									
	Found	23	32	0	0	0	13	17	0
	Expected	27.5	27.5	0	0	0	15	15	0

* The deficit of albinos in F_2 and $F_1 \times 13C$ is possibly real (exceeds 5 percent level of significance).

† Expectations are based on the assumptions that the albino stock 13C is *cchhAA*, the black self stock M is *CCHHaa*, and there is no linkage.

‡ The albino count was made at birth; classification of colored rats was made at weaning and thus involves a smaller number because of litter reduction.

generally mated the two best females of each litter to the best male of the chosen litter for mating for ten days, and then after a two-day interval mated them to the second best male, so that if the chosen best is sterile or slow breeding, the second best can be used without loss of time. Until recently the litter size was large enough (10 to 20) to allow the scheme to be carried through on the basis of one litter per pair, litters being reduced to eight shortly after birth in random fashion. In this way we have gotten nearly three generations per year, with both strains coming to breeding age at the same time. Recently, however, litter size in the small line has fallen below eight so that second litters are needed, causing some delay. The unselected 4St stock was not carried along to serve as control, and this is a flaw in the experiment. We do, however, have observations in the 13M strain that is not under size selection pressure and carried on with a comparable effective population size.

RESULTS

Body size selection

Animals were weighed at weekly or semiweekly intervals throughout their lives, and the resulting growth data will be discussed elsewhere. For present purposes of describing the progress of size selection we will use simply the weight at nine weeks, which was the principal determinant in selection. There is a large sex difference in rat growth; unlike the case with mice, the male grows *relatively* (as well as absolutely) much faster than the female, so that the ratio of male to female weight increases rapidly with age. This presents a problem in combining male and female size data. We have had a considerable degree of success in fitting *individual* as well as average postweaning growth curves with the equations

$\log W = \log W\infty - 3.65/\text{age (weeks)}$ for males

$\log W = \log W\infty - 2.73/\text{age (weeks)}$ for females

with $W\infty$ (weight at $1/\text{age} = 0$, or age $= \infty$), being the sole adjustable quantity for any individual curve (L. M. ZUCKER 1942, 1953). The equations illustrate the fact that weight ratios within the same sex remain constant with age, while the ratio of male to female weight increases continuously with age, being made up of the limiting weight ratio and an exponential factor containing the age and the difference between the characteristic male and female slopes (3.65–2.73). In groups of rats of very different average sizes, we find a constant ratio of male to female weight at nine weeks, with the value very close to 1.45. We will then assume that any observed female weight at nine weeks of age, when multiplied by 1.45, supplies the weight which the rat would have shown had it been a male. Female weights, so corrected, are averaged with male weights to give the average nine-week weight for any group.

Results are presented in Figure 1 in three different forms. Section A shows the ratio of mean nine-week weights in the two selected lines, plotted against generation number. This is a satisfactorily smooth curve, representing steady progress in selection. Section B shows separate plots for the two lines against calendar time together with a control strain (13M) not subjected to size selection. Section

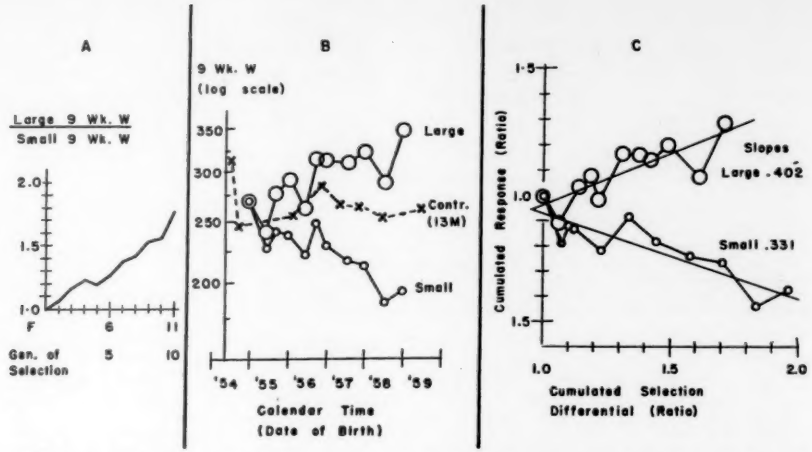

FIGURE 1.— Progress of body size selection. A. Ratio of nine-week weights of large and small strains against generation of selection. B. Separate plots of nine-week weights for large and small lines, and control line not selected for body size. Abscissa is calendar time. The first points for both 4St and 13M may possibly be high due to heterosis. The weight scale used is logarithmic. We have used ratios, or logs, or CV's throughout rather than differences or absolute weights or variance of absolute weights. The standard deviation tends to be proportional to mean weight, to be more constant on *log W* scale (cf. similar spreads of weight histograms in Figure 2B where successive class means are in geometric progression.) The ratio, not difference, of weights of two well-nourished and healthy individuals or groups of the same sex but different size remain constant during postweaning life. At any age the ratio, not difference, of male and female weights is constant. Consideration of ratios rather than absolute differences make much more biological sense.

C. Cumulated selection differential (calculated as weight *ratio*, not difference) against cumulated response (as weight ratio). This corrects the plot of section B for effects of more favorable selection differential in one line than in the other (as suggested by FALCONER 1953b). The slopes are realized heritability, and were calculated from the regression of response on selection differential.

C shows the same data in the form suggested by FALCONER (1953b)—cumulated response against cumulated selection differential. Sections B and C show marked irregularities not found in A, which must be due to environmental variation affecting both strains alike. The most prominent effect is that of going through the most rapid growth period during the hot summer weather (New York City, no air cooling). The summer of 1957 was relatively cool and free of long hot spells, and the graph shows it. Under these conditions it is not surprising that the coefficient of variation of the nine-week weight is highly variable and rather uninterpretable (see Table 3).

The slope of the plot made in section C of Figure 1 represents realized heritability. Slopes for the two lines are about the same. This is unlike the experience with mice, where selection down is more effective than selection up. In our first size selection experiment in rats, with the initial stock definitely smaller in size

TABLE 3

Coefficient of variation for nine-week body weight in control line and selected lines

	13M					4StS				4StL			
	Female		Male			Female		Male		Female		Male	
	CV*	N	CV	N		CV	N	CV	N	CV	N	CV	N
F_{3-4}	8.0	91	9.5	82	F_{3-5}	9.9	101	11.2	120	6.9	97	6.6	98
F_{5-6}	6.4	86	6.8	73	F_{6-8}	7.1	117	9.2	121	7.4	78	7.3	87
F_{7-8}	7.5	67	10.0	64	F_{9-11}	9.2	90	9.5	85	9.7	105	8.9	102

* CV's as calculated for each generation are extremely variable. We have included all available rats, some of them not in the main line of selection (i.e., litters of which one parent was second choice), in order to get as large numbers as possible. For further smoothing, we have averaged CV's for two or three successive generations, comprising in each case approximately one calendar year. All data on one line are approximately contemporary.

(nine-week weight about 230) than 4St, selection up was very much more successful than selection down (unpublished data). Two important factors contributing to asymmetry in two-way selection are dominance and the position of the initial phenotype in relation to the possible extreme phenotypic values (FALCONER 1953b). Dominance always favors selection towards the recessive. The effect of the initial phenotypic position is to favor selection towards the further phenotypic extreme and inhibit selection towards the nearer extreme. This effect, unlike dominance, can therefore cause reversal in relative rates of up and down selection as the initial phenotypic value in different selection experiments varies. Since, in mice and rats subjected to size selection, symmetry and opposite asymmetries have all been observed, it seems likely that the position of the initial phenotype in relation to the extremes has been an important variable, whatever the dominance situation may be.

Size of hood

CASTLE and co-workers (see reviews in CASTLE 1940, 1951) have shown that the size of the hood in *hh* rats responds readily to two-way selection, leading to two lines with very large and very small hoods, with ranges outside the range of the original stock. The genetic variability was not completely exhausted even by 20 generations of intensive selection. It was first concluded that "the genetic modification secured by selection did not involve the hooded gene itself but only the residual heredity (consisting probably of an association of genes which modified the somatic effect of the hooded gene itself)." There was later some evidence that eventually the selection also profited from a change at the hooded locus itself, with the appearance of a new allele h^i (true Irish) in the plus line, and possibly of the new allele h^n (notch) in the minus line.

Presumably in selection for body size one also makes progress by selecting for favorable configurations at many loci which modify by contributing plus or minus factors to the basic character, the rat physique. No doubt, body size selection will also concentrate the most favorable alleles of any possible allelic series affecting size, whether originally present in the population gene pool, or arising

by mutation during the course of selection. At any rate the two characters, body size and hood size, are alike in their continued response for many generations to two-way selection and in the conceptual structure which can be built up around the observed facts. It is of interest to see whether selection for the one character has any influence on the other.

Observations have been of two kinds. (1) Hood size was classified in four degrees for the first five and for the tenth generations. There has been no change of the average in either line. (2) In the tenth generation (ninth of body size selection), careful measurements were made of hood size in the two lines. As described by WRIGHT and CHASE (1936), a drawing was made within a rubber stamp outline, one for the dorsal and one for the ventral aspect. This was done by one person, and when the rats were four- to six- weeks old. Areas were measured by a planimeter. Two different areas were measured—the area of the colored stripe down the back starting at the edge of the band of solid color covering head and shoulders, and the total area of color over the whole body. The second measure includes a large nearly constant colored area, and the total is principally modified by variation in the first measurement—the stripe itself—although there is also some variation in the amount of white on the forelegs and in occasional small colored spots which show on the belly. Results with the two areas were very similar in sense. Figure 2 shows the distribution of measurements of the stripe area, compared with the distribution of nine-week body weights for the two lines. It is clear that selection for body size is having little if any effect on hood size; the only effect is the greater dispersion of hood size in the large line, with two new classes added to the histogram. At the present stage of selection there is no real difference between the two lines in respect to hood size.

One view of quantitative characters is that they are determined by a network of gene loci, very large in number and probably spread over all or many chromosomes. A common or even largely common set of such loci for our two characters is clearly ruled out. Two independent sets of genes, if each is numerous and widely distributed, would certainly show some linkage. Here the expected behavior under size selection is a little uncertain. The foundation stock cannot have been in full linkage equilibrium, but it is impossible to say how far out of linkage equilibrium it was. This depends on the extent to which pairs of linked loci were oppositely fixed or had very different allelic frequencies (i.e., were nearly oppositely fixed) in the different parent stocks. If the foundation stock was out of linkage equilibrium for many relevant loci, one would expect a gradual approach to equilibrium during the course of generations with some establishment of differences in hood size in the two lines, due to such linkage in the early stages of size selection, followed by no further change after some later stage. But our failure to find such an early difference in hood size does not rule out the possibility that both characters are controlled by large overlapping networks of genes; it only rules out the idea of a single set of pleiotropic genes principally determining both characters.

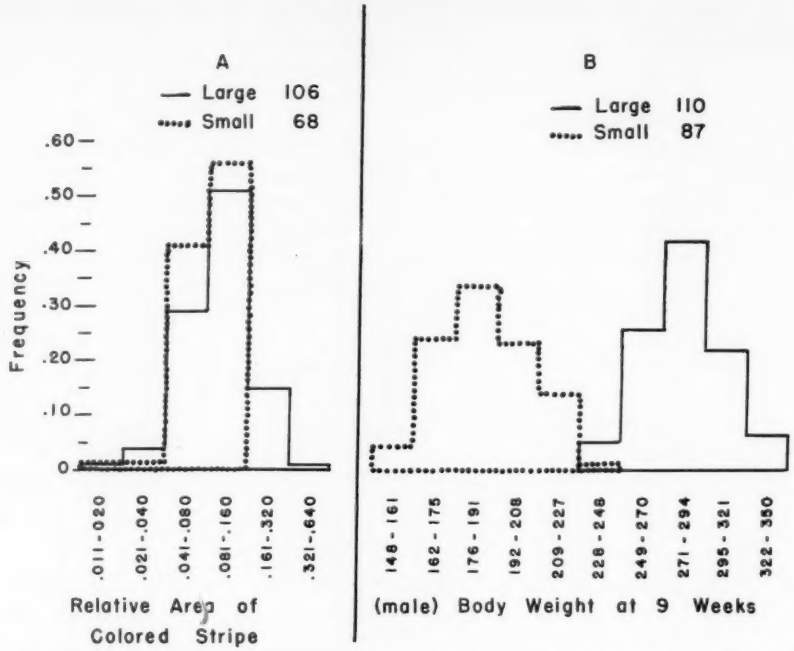

FIGURE 2.—Effect of nine generations of two-way selection for body size on size of colored hood. Our six classes of stripe area can be compared to the scale used by CASTLE and PHILIPS (see CASTLE 1940) as follows:

Our class	1	2	3	4	5	6
CASTLE and PHILIPS	—0.5, 0	0.5	1	1.5	2	2.5, 3, 3.5

Three major coat color genes

In contrast to the relative stability of the hood size in hooded rats during selection for body size, we find large changes in coat color pattern. The large line is now essentially restricted to albinos and black hooded rats; the small line has practically no albinos, has appreciable numbers of agoutis, has also self rats, and has large numbers of pseudo-Irish. Altogether the small line has much more color than the large one. MACARTHUR (1949) observed large net changes in color during his size selection experiment and suggested as possible explanations either chance (random drift), or a possible true association, the latter especially likely in view of other evidence from crosses that two of the genes concerned—brown and dilute—are associated with body size (GREEN 1931; CASTLE 1941; for objections BUTLER 1954). However, CASTLE had concluded from similar experiments that albino and agouti are not associated with size, and when our data are analyzed in terms of changes at the three relevant gene loci, the changes appear to be probably of chance origin.

Calculation of allelic frequencies: Initial values of all three allelic frequencies q_c, q_h, and q_a are known from the constitutions of the four stocks crossed. Subsequently, exact q's can be calculated only if the heterozygote is distinguishable from both homozygotes, and this is true only for the hooded locus. Table 4 shows successive values in the two lines for q_h calculated as the frequency of hooded rats plus half the frequency of pseudo-Irish. Table 4 also includes approximate values for q_h, from the square root of the frequency of hooded individuals. This approximate calculation assumes Hardy Weinberg equilibrium—i.e., random breeding. It is apparent that this is a very good approximation, although there is a slight tendency for the approximate calculation to be too low; we shall come back to this small discrepancy later. The close approach to Hardy Weinberg equilibrium in the population for one locus means that it should hold for the other two loci; this gives us a method for calculating q_c and q_a.

Genetic drift and gene fixation: Figure 3 shows the course of the three allelic frequencies during size selection. A character associated with body size would be

TABLE 4

Frequency of hooded allele h

F no.	2	3	4	5	6	7	8	9	10	11	12	
Selection no.	1	2	3	4	5	6	7	8	9	10	11	
4StS												
	.78	.74	.72	.53	.59	.64	.69	.75	.76	.72	.78	exact
	.74	.71	.73	.50	.56	.59	.66	.71	.79	.71	.78	approx.
4StL												
	.76	.80	.79	.81	.83	.93	.92	.95	1.00	1.00	1.00	exact
	.77	.77	.78	.78	.85	.93	.91	.94	1.00	1.00	1.00	approx.

Exact calculation: $q=[hh]+\frac{1}{2}[hH]$.
Approximate calculation, assuming Hardy Weinberg equilibrium: $q=\sqrt{[hh]}$.

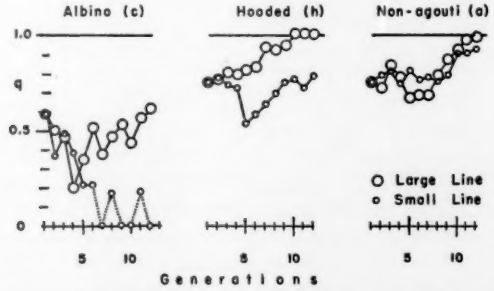

FIGURE 3.—Changes in three coat color genes during body size selection with 12 breeders per generation. For alleles c and a, q is calculated by an approximation which deteriorates rapidly in the neighborhood of $q = 0$; hence, the broken lines and repeated apparent but not real fixation of c in the small line.

expected to move in opposite directions in the two lines, and it appears that there are no continuing opposite trends. The possibilities for large cumulative genotypic changes due to chance—genetic drift—are considerable in small populations (CROW 1954; LI 1955, p. 316 *et seq.*). In the present case we are drawing a sample of 12 out of the possible infinite offspring population, and each individual is very poorly able to represent the average q of that infinite population, since q's for individuals can be only 0, 0.5 or 1. Therefore, a group of 12 is quite unlikely to give the same q as the previous group of 12. If the sampling error chances to go in the same direction for several generations there is drift, and long continued drift can lead to gene fixation. In the size selection experiment one of the six genes is probably fixed (h in 4StL, in doubt because of the possibility of albinos carrying H), and others are very close to being fixed. But if h were fixed in 4StL because of obligate association with size, one would certainly expect rather different behavior in 4StS than that shown.

As will be seen below (Table 5), the probability due to genetic drift of fixation

TABLE 5

Results of model breeding experiment replicated 120 times

Generation number*			F	$\overline{F}_t$	q	σ_q^2	$(F-\overline{F}_t)q(1-q)$	Number of homozygous lines	
								gg	*GG*
Inf. pop.	*gg*=.56; *gG*=.38; *GG*=.06		—.013†	...	.75	...	...	.	.
Parent	6F, 6M drawn from above, mated at random		—.002	—.039	.745	.0063	.007	0	0
1	6F, 6M offspring of above, mated to minimize inbreed		—.009	—.062	.747	.0113	.010	0	0
2	"	"	—.042	—.113	.741	.0145	.014	3	0
3	"	"	—.056	—.136	.748	.0171	.015	3	0
4	"	"	—.018	—.116	.747	.0215	.019	4	0
5	"	"	.067	—.092	.749	.0261	.030	7	0
6	"	"	.081	—.065	.749	.0296	.028	9	0
7	"	"	.085	—.106	.744	.0351	.036	13	0
8	"	"	.106	—.104	.741	.0393	.040	14	0
9	"	"	.172	—.030	.745	.0420	.038	16	0
10	"	"	.159	—.086	.743	.0453	.046	16	0
11	"	"	.183	—.093	.743	.0484	.052	18	0
12	"	"	.187	—.100	.737	.0560	.055	21	1
13	"	"	.223	—.124	.738	.0610	.071	24	1
∞			1.000			.189		90	30

* Generation number comparable to the number of generations of selection for 4St, and one less than the F number for 4St.

† Not zero, as it should be for an infinite population; the frequencies of the three genotypes were rounded off to two digits for convenience.

Symbols: Quantities calculated for a subpopulation are labelled with a subscript—q_t, $[gg]_t$, F_t. The average of these quantities for all the subpopulations is shown by a bar—$\overline{q_t}$, $\overline{[gg]_t}$, $\overline{F}_t$. Since the inbreeding coefficient is indeterminate for homozygous lines, $\overline{F}_t$ includes only unfixed lines whereas the other quantities are calculated for all the lines. Quantities calculated for the over-all large population obtained by pooling all the subpopulations are shown with plain symbols—q, $[gg]$, F. $q=\overline{q_t}$; $[gg]=\overline{[gg]_t}$; but $F \neq \overline{F}_t$.

of one gene by the 11th generation of this breeding scheme and an initial q of 0.75, is 0.154, so that in sets of four trials (corresponding to our four rat cases with initial q of 0.75), the expectation would be

none fixed	1 fixed	2 fixed	3 fixed	4 fixed
0.512	0.373	0.102	0.012	0.000

The rat results—one out of four probably fixed—are in good agreement with this expectation.

Expectation for fixation at the albino locus, with initial $q = 0.58$, would be less because the starting point is farther from either end. The near fixation in one out of two trials as has developed in the 4St breeding experiment is, therefore, not the most probable finding, although quite possible. However, in the data of the 13C × M cross (Table 2) there is a just significant deficit of albinos, suggesting that in one, at least, of the original albino stocks *c* may be linked to a gene unfavorable for early survival. If this were the case, it would also explain the fact that in the first four generations of selection albinism decreases rapidly in both lines. As the populations approach equilibrium with regard to this linkage, drift can send the albinism back up again, as in the 4StL. Or conceivably, albinism might even yet turn out to be associated with large body size, a relation obscured by an opposing linkage phenomenon; opposite trends have indeed been maintained since the fourth generation. It is of course also possible that for the other loci there is some partial association with size which is so far obscured by genetic drift operating to oppose the directed change.

Inbreeding and outbreeding effects: The fact that in Table 4 the approximate calculation for q_h is usually slightly below the exact one is a reflection of the fact that these populations usually deviate from Hardy Weinberg equilibrium by showing a slight *deficit* of homozygotes and an excess of heterozygotes. The apparent inbreeding coefficient calculated for the hooded locus is about -0.1 (corresponding to a difference between the two calculations of Table 4 of 0.01, 0.02 or 0.025 for q of 0.1 or 0.9, 0.7 or 0.3, and 0.5 respectively). The inbreeding coefficient for this breeding plan should be positive, rising by about 0.02 per generation. The reason for this apparent discrepancy is twofold; the first element in the discrepancy is the fact that the expectation of a positive F applies to a statistically large population made up of many subpopulations each carried on by this breeding plan, and not to any subpopulation alone.

It has been shown (WAHLUND; see account in LI 1955, p. 297 *et seq.*) that small subpopulations each carried on with random breeding (subpopulation F_i averaging zero) combine into large populations in which the over-all F rises steadily with generation number; the value of F depends on the spread in q_i values of the subpopulations, which in turn depends on the effective subpopulation size. Then the average subpopulation inbreeding coefficient $\overline{F}_i$ (each one calculated in relation to the corresponding q_i) remains zero, but the over-all population inbreeding coefficient F (calculated in relation to the overall q, or $\overline{q}_i$)

rises steadily. There is, therefore, no inconsistency between an expected positive F and observed $\overline{F}_i$ of zero.

However, our F_i values average near -0.1. This deviation from Hardy Weinberg equilibrium cannot be explained by any hypothetical linkage to genes under selection. In the selected breeder population one can of course expect deviations from equilibrium with regard to genes under selection, and therefore with regard to any linked genes not yet in linkage equilibrium. But no matter how distorted the parent population, the offspring population must be in Hardy Weinberg equilibrium with regard to each gene, provided the selected rats are mated at random. It is the use of non random mating which produces the observed Hardy Weinberg disequilibrium in the offspring population, and this statement will now be verified in a model experiment involving the same breeding plan, but no selection and no linkage.

Model breeding experiment

The breeding scheme employed in the rat experiment involves two necessary consequences of small population size (CROW 1954): (1) the piling up of common ancestors and (2) large random sampling error, or (1) inbreeding and (2) genetic drift, together with a third feature, the mating of the most distantly related, which was introduced to minimize inbreeding effects. The coat color data illustrate the effects of sampling error and of mating the most distantly related, but are incapable of illustrating the effects of the piling up of common ancestors and the interplay of this feature with the others. To illustrate this interplay more completely, and to obtain readily some quantitative expectations for the behavior in our rat subpopulations, we have carried out a model breeding experiment. The model is a single gene locus with the random feature supplied by a table of random numbers and the breeding plan as in the rat experiment except that there is no selection. This is a proper model for our results on the coat color genes, assuming there was no (direct or indirect) selection for these. The model experiment was repeated 120 times. Then we can see empirically how inbreeding coefficient, gene equilibrium and genetic drift operate both in subpopulations and in the total population.

Procedure: Let us start with an allelic frequency $q_0 = 0.75$ (where g and G are the two alleles at a locus) and with an equilibrium population. Then the frequencies of the three genotypes gg, gG and GG are (in two-digit numbers) 0.56, 0.38 and 0.06. Let us assign the numbers one through 56 to genotype gg, 57 through 94 to gG, 95 through 00 to GG; draw 12 two-digit numbers from a random number table and write out the corresponding 12 genotypes. This gives us the first 12 breeders; we will call the first six females, the last six males, and mate one with seven, two with eight etc., to get a random mating. Matings $gg \times gg$, $GG \times gg$ and $GG \times GG$ have only one kind of offspring. In $gG \times gG$, assign the numbers one and two to gg offspring, three through six to gG, seven and eight to GG; draw two one-digit numbers from the table, rejecting nine and zero, and the two corresponding genotypes are two random offspring from this mating. For

matings $gg \times gG$ or $GG \times gG$, assign numbers one through four to one type of offspring, four through eight to the other, and proceed as before. In this way one can set up the six matings in each generation and draw at random the two offspring from each pair, which constitute the 12 breeders of the next generation, and so on as long as one likes. As in the rat experiment, the most distantly related individuals are mated. The allelic frequency for each generation is that of the 12 parents of that generation, the value which would also be found in a large population composed of equal numbers of offspring of those 12 parents; it differs by sampling error from the allelic frequency of the actual 12 individuals drawn from this infinite population.

Model breeding experiments to illustrate effects of close inbreeding, using a random number table in this way, have been published by BONNIER (1955). We were not aware of this until after we had done a good deal of work with our procedure.

Results: Table 5 summarizes the results in terms of inbreeding coefficients (for total population and average for the 120 subpopulations), allelic frequency and its variance, and gene fixation. It seemed advisable to check the procedure by comparing the results with theory where possible. Figure 4 compares the findings with expectation for the variance of allelic frequencies and for population inbreeding coefficient, or rather, the inverse function heterozygosis ($1\text{-}F$). The expected steady increase in variance of q (corresponding to drift in the individual q's) begins at once because the sampling error is present in each generation. The expected decrease in heterozygosis due to breeding from small population begins only after the fourth generation, when the 16 great great grandparents required for each rat must have been supplied by the 12 individuals of the first set, so that some were used twice. Up to this point there were no common ancestors; therefore, no loss of heterozygosis on this account; and, in fact, there is in the early generations an actual rise in heterozygosis (negative F) due to the avoidance of matings of close relatives. Thus we have for a few generations inbreeding and dispersion of q effects dissociated from each other in sense. CROW (1954) has discussed another case in which these two generally related effects of small population size can diverge from each other.

As in the rat experiment, the average subpopulation inbreeding coefficient $\overline{F_i}$ fluctuates around -0.1 (actual average -0.09), which presumably simply reflects the avoidance of mating of close relatives in the preceding generations and is not cumulative. Within each subpopulation there is a noncumulating relative excess of heterozygotes in relation to the q_i for that subpopulation, while in the total population there is a steadily growing deficit of heterozygotes in relation to the pooled q for the entire population.

Inbreeding coefficients, based on breeding history, apply to large populations, not to subpopulations; but they also apply to individuals with many gene loci. We can consider the 120 replicate breeding experiments as one experiment on a model in which each individual has 120 gene loci. Considering this pool of 120 genes, the individual with a history of inbreeding—a positive inbreeding co-

efficient due to common ancestors—has relatively more loci in a homozygous condition than would be expected by chance.

Unexpectedly it was found (cf. Table 5) that

$$\sigma_q^2 \cong (F-\overline{F_i})q(1-q)$$

This looks like an extension of the equation (WRIGHT, WAHLUND; see LI 1955, p. 298) for subpopulations of small size within which breeding is fully random,

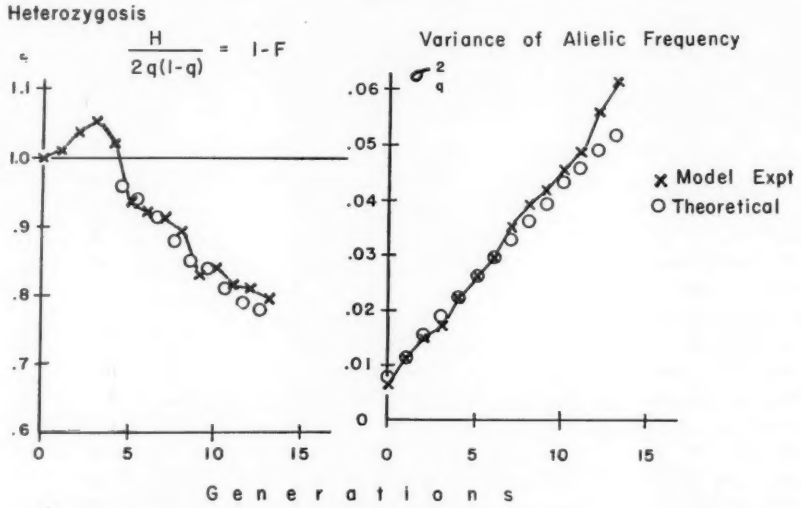

FIGURE 4.—Comparison of empirical model with theory.

Heterozygosis: theoretical values calculated as the average of the predictions for 16 and for eight breeders, when only the most distantly related are mated (WRIGHT; see LI 1955, p. 209, Table 59). The quantity plotted is $H_t/2q(1-q)$. Results are not plotted against generation number because the common ancestry which lowers heterozygosis makes a first partial appearance in the fourth generation (four of the 12 individuals of F_0 each provide two great great grandparents), and a more complete appearance in the fifth generation *et seq.* (where all 12 individuals of the $(t\text{-}4)$th generation appear more than once as an ancestor in each individual's ancestry); therefore, the calculated values are plotted midway between 4th and 5th, then 5th and 6th, etc. The values for the model were calculated as $(1\text{-}F)$ from the data of Table 5.

Variance of q: Theoretical values were calculated from the equation given by CROW (1954)

$$\sigma^2_{q_t} = \frac{q(1-q)}{2N_e} + \left(1-\frac{1}{2N_e}\right)\sigma^2_{q_{t-1}}$$

where N_e is the effective population number. For the first value, representing 12 individuals drawn from an infinite population, the second term vanishes, and N_e is 12—i.e., $2N_e$ is 24. For all subsequent values however the effective population number is twice the number of breeders, so that $2N_e$ is 48, because each breeding pair contributes two offspring to the population, rather than the offspring contributions per pair following a Poisson distribution. (CROW 1954).

so that $\overline{F_i}$ would be zero. This equation is: $\sigma_q^2 = Fq(1-q)$, and is readily derived:

$$Fq(1-q) = [gg] - q^2 = \frac{\sum_i [gg]i}{N} - q^2$$

For random breeding in the N subpopulations, $[gg]_i = q_i^2$, so $Fq(1-q) = \frac{\sum_i q_i^2}{N} - q^2$ and this is the same as variance of q_i if we ignore the difference between N and $N-1$. If one tries to carry out a similar derivation for the extended equation by writing out the values of F and of the F_i's in terms of q and the q_i's, this does not reduce to the variance of q without two quite impossible simplifications. The equation must, therefore, rest on some other derivation, possibly involving a relation between inbreeding coefficient of the offspring and the parent q or variance of q. (It should be noted that since F is indeterminate in value for a homozygous population, $\overline{F_i}$ is calculated for only those lines not yet fixed, whereas F, q and variance of q include all lines.) The relation is certainly very closely true in this experiment and applies equally well to a similar model breeding experiment with the same breeding scheme but with two-way selection (L. M. ZUCKER 1960), where in one line fixation finally reaches 100 percent with the observed relation continuing to apply up to the end.

Gene fixation amounts to 18 out of 120 cases by the eleventh generation (see Table 5). Obviously the immediate basis for this is the genetic drift, not the inbreeding (common ancestors) *per se*. Fixation has already started by the second generation when the heterozygosis in the general population is greater than one; fixation proceeds steadily during the next three generations at which point, for the first time, inbreeding—loss of heterozygosis makes its appearance.

For each gene there is random drift of the allelic frequency until it gets too close to one or zero. Although eventually, when all genes are fixed, a fourth of the loci will be fixed *GG* and three fourths *gg* (because initially $q_g = \frac{3}{4}$), the early fixations are all necessarily *gg* because the starting point for the drift is so much closer to one than to zero. Actually drift towards lower q is faster than drift towards one; by the eleventh generation, when 18 have been fixed *gg* (i.e., have at some time reached $q = 1$), 27 loci have at some time reached 0.5, an equal distance in the other direction, but of course they are not caught at $q = 0.5$. The larger number moving to 0.5 than to one is probably a real difference. The possible rate of drift differs with the type of mating; thus $gG \times gG$ is the most favorable for drift, since in extreme cases the offspring can differ by 0.5 unit of q from the parents, while matings of heterozygote with homozygote allow a maximal change of only 0.25. Thus the rate of drift should be greatest when q is near 0.5, while asymmetry of gene fixation is greatest when q is near one end.

Evidence of obligate association between characters

The terms satellite or correlated characters have been used for characters which concentrate in a line being selected for some other character. If such a satellite character is due to a single gene, and the population size is small, the association

may be purely a chance one, due to genetic drift. If, however, one is dealing with a truly polygenic character, determined by equivalent contributions from many loci, with a plus contribution from one locus capable of neutralizing a minus contribution from another, such a character should be quite stable under the effects of genetic drift. Hood size is possibly such a character. Obligate association between two such characters should be readily obvious without confusion from drift. (If such a character starts near the extreme end of its range, however, a slow net drift away from the end is likely. This is because drift towards the near end cannot be as numerically large as drift towards the center. This effect is possibly illustrated by the slight downward trend of q in Table 5.) One can also readily envisage a type of character dependent on many loci where a favorable change at one locus cannot counterbalance an unfavorable change at another locus. Fertility is probably an example. Unfavorable drift at any one locus tends to spoil the character irrespective of favorable changes at other loci. Such characters should be practically as much subject to genetic drift in small populations as are unit characters. It is, therefore, usually necessary to get independent evidence that an apparent satellite character is obligately so, either by a repetition of the selection experiment *de novo*, or even better, by selecting for the satellite character to see whether the other character will then also be concentrated (SOKAL and HUNTER 1954; WEIR *et al.* 1953). We will report elsewhere on several characters associated with body size in two independent size selection experiments.

SUMMARY

A two-way size selection experiment is being carried out on a rat stock heterozygous for three major coat color genes and an unknown large number of genes modifying the size of the colored area in hooded rats. After ten generations of within-litter selection there is a nearly 80 percent difference in mean body size, with rate of progress in the two directions about the same. So far there is no evidence of correlation between any coat color character and body size.

The three single gene loci are subject to marked genetic drift owing to the small population size (12; effective size 24), with one locus probably fixed and others near to fixation.

An empirical model illustrating the genetic drift and closely matched to the actual rat breeding plan has been set up and replicated 120 times, allowing the calculation of inbreeding coefficient within subpopulations and for the over-all population, genetic drift as measured by the variance of the allelic frequencies, rate of gene fixation, etc., all of which agree well with relevant theory. From an initial allelic frequency of 0.75 the probability of fixation of a gene rises about 0.016 per generation, with the first 17 percent being fixed at the near end of the range of q. The results of the rat experiment are in good agreement.

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MODEL BREEDING EXPERIMENT FOR TWO-WAY SELECTION IN SMALL POPULATIONS SHOWING DIRECTIONAL DOMINANCE[1]

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Received September 30, 1959

WE have described a procedure well-suited to small populations and multigenic subjects for carrying out model breeding experiments on paper (L. M. ZUCKER 1960). In the model experiment carried out, there was excellent illustration of various known effects of breeding without selection in small populations over many generations; loss of heterozygosis and random drift behaved in accordance with theoretical expectation. Now we wish to present another model experiment concerned with the effect of directional dominance on two-way selection in small populations for a character determined by 40 gene loci and large nongenetic variability. Here the course of random drift and loss of heterozygosis deviate considerably from theoretical expectation. Also, with regard to gene fixation and relative phenotypic progress in the two directions, the results are much closer to what would be found for a dominant major gene than was expected for such a polygenic system with large nongenetic variability.

FALCONER (1953) has discussed the effect of directional dominance on two-way selection of characters subject to large nongenetic variability. While directional dominance is a potent source of asymmetry—i.e., difference between the rates of progress and realized heritability in the two directions of selection—he concluded that there will be symmetry even with directional dominance if the initial phenotype is midway between the extreme limit values. The model breeding experiment to be reported here was designed to fit this condition, although differing from his theoretical model in having relatively small population size (12 breeders per generation, effective population size 24), and in using within-litter selection rather than mass selection. The result has been a great selective advantage for the recessive with rapid phenotypic progress and complete fixation of all 40 genes, whereas selection towards the dominant led to much slower phenotypic progress and little gene fixation.

The results have been privately criticized as not valid in general because they represent a single experiment which ought to be repeated many times. Since the paper work is very laborious, multireplication in this way was not feasible, but the procedure should be quite easily adapted to a digital computer. However, owing to various circumstances including a move from one location to another (after arrangements had been made at the first location to use a computer), the

[1] This investigation was supported by research grants C-2471 and C-4241 from the National Institutes of Health.

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further working out of this project has been repeatedly delayed, and in the meantime other projects in progress have come to our attention.

Actually, the model contains 40 equivalent gene loci; with regard to rate of gene fixation the single experiment with 40 genes is quite equivalent to 40 experiments with one gene. Even with regard to the quantitative character itself—the over-all phenotypic value resulting from the action of 40 genes and nongenetic variation—while this is a single experiment without replication, it is no more vulnerable in this respect than any single experiment on living forms, and the model experiment allows one to inspect the inner workings and test out possible explanations. Since the experiment is free of complications due to gene-gene or gene-environment interactions, which are powerful concepts to which one often appeals in actual experiments for explanations of the unexpected, we are forced to look for the explanation within the limited features of the model itself—directional dominance, small population size, within-litter selection.

PROCEDURE

We assume 40 genes, each showing full dominance and contributing 0.25 to the phenotype when *gG* or *GG*, zero when *gg;* phenotypic extremes are thus zero and ten. The scale unit is the standard deviation of the nongenetic variation. The breeding scheme is that used by FALCONER in his mouse selection experiments and in our previous report. In each generation there are six pairs of breeders each of which has eight offspring, four of each sex; the best female and the best male of each litter comprise the new breeder population representing a selection of 25 percent. In each generation the most distantly related are mated.

Configurations at each of the 40 loci of the initial 12 breeders were determined by drawing two alleles per breeder from an infinite population with allelic frequency $q_g = 0.7$ by means of a random number table (numbers 1-7 represent allele *g*, 8-0 allele *G*. We might have gotten an initial population more representative of the assumed initial conditions if we had used the procedure of our previous report where, instead of drawing alleles from an infinite population of fixed q, we drew genotypes from an infinite population with $[gg] = q^2$, $[gG] = 2q(1-q)$, and $[GG] = (1-q)^2$). The procedure used, when repeated 40 times, gave the configurations at all 40 loci for the initial 12 breeders which were then mated at random. The choice of average q at 0.7 puts the initial phenotype in the middle of the range. The configuration at each locus for the eight offspring of each mating was determined by the mating type and the use of a random number table as previously described.

A necessary part of this model, differing from that of the previous report, is variability of nongenetic origin. We assumed an environmental variation with $\sigma = 1$ applying alike to all genotypes. The area under a normal distribution curve with $\sigma = 1$ was divided into 100 equal areas, and the center ordinates of these 100 areas represent 100 equally probable errors, half +, half −. To these were assigned two-digit numbers from 00 to 99. For each individual offspring a two-

digit number drawn from the random number table gave its appropriate nongenetic error. Error-free phenotypic values ($Phen_g$) were calculated for each offspring as $(40 - gg) \times 0.25$, where gg is the number of loci in that individual with gg configuration; this value was corrected to the final value $Phen_{g+e}$ by the addition or subtraction of the random nongenetic error. Female sex was assigned at random to four individuals in each litter. Selection could then be made for the apparently largest female and male in each litter (or smallest), and the mating for the next generation could then be set up.

RESULTS

The model as carried through provides the complete genotypic composition of each generation, both of the selected population of 12 breeders and of the unselected population of 48; from this, one can calculate any desired population quantity. Obviously not all of this material can be reported in detail. We have chosen to tabulate (Table 1) for many of the generations observed, the number of homozygous lines of each type; the population inbreeding coefficient F (which measures the relative deficit of the heterozygote in the pooled 40 gene unselected population); the average of the inbreeding coefficients F_i calculated separately for each gene locus $1, 2, \ldots i \ldots 40$ (which measure the relative deficit or excess of the heterozygote at each of the 40 loci in the unselected population); the frequency q of allele g in the pooled 40 gene unselected population (and q is the same as the average of the 40 individual q_i's); the variance σ_q^2 (which measures the steadily rising dispersion of the q_i's due to random drift); and finally the average phenotypic values for selected and unselected populations, $Phen_g$ being calculated from the genotypic distribution, $Phen_{g+e}$ being calculated as the combined phenotypic expression of the genotypes and the nongenetic error. Selection was of course based on the value of $Phen_{g+e}$, the only one of these quantities ordinarily available in an actual experiment. Some additional information is provided in Figure 1, which shows the actual distribution of the 40 values of q_i at a few chosen generations, and in Figure 2A which includes a plot of the course of phenotypic progress. The experiment was originally planned for 20 generations, but the down selection was extended to complete gene fixation (37 generations).

As will be seen from Table 1, columns 2 and 3, gene fixation proceeds rapidly in down selection, hardly at all in up selection. Table 1, column 10, and Figure 2A (large circles) show the asymmetry in phenotypic progress favoring down selection. Table 1, columns 4 and 6 show steady rises in inbreeding coefficient and spread in q_i values. Such changes are expected with moderate inbreeding (small population size); however, it will be seen that the inbreeding coefficient F rises much more rapidly in the down selection than in the up selection, as though selection towards the recessive were imposing additional signs of inbreeding beyond what would be expected from the pedigree.

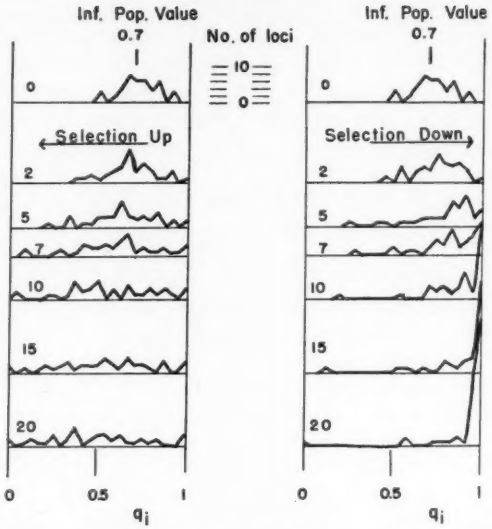

FIGURE 1.—Effect of combined selection and random drift on the frequencies of the recessive allele at the 40 loci. Each graph represents number of loci plotted against allelic frequency, and one moves down the figure with rising generation number. Selection up is for large phenotypic values, low values of q.

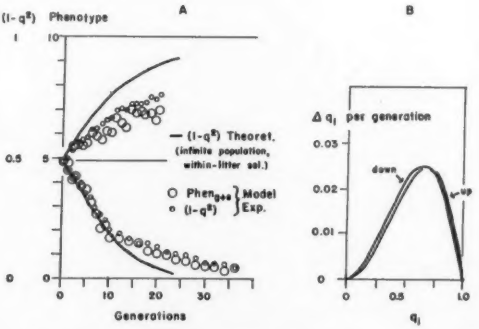

FIGURE 2.—Comparison of the results of the model with theory. In 2A, large circles represent $Phen_{g+e}$ (column 10, Table 1); small circles represent $(1-q^2)$ (from q listed in column 6, Table 1). The difference between large and small circles represents inbreeding depression. The nearly symmetrical heavy curves represent theoretical expectation for the progress of $(1-q^2)$ for conditions like those of the model except for population size (equations shown in Table 2). Figure 2B shows the expected change in q per generation of selection as a function of initial q (from the same equations).

TABLE 1

Summary of results

1 Gen. no.	2 Homozyg. lines-N gg	3 GG	4 F	5 $\overline{F_i}$	6 q	7 σ_q^2	8 $(F-\overline{F_i})q(1-q)$	9 Unsel pop. $Phen_g$	10 Unsel pop. $Phen_{g+e}$	11 Sel. pop. $Phen_g$	12 Sel. pop. $Phen_{g+e}$
Selection down*											
0	0	0	.064	—.001	.718	.0117	.013	4.72	4.93	4.35	3.61
1	1	0	—.036	—.108	.735	.0130	.014	4.57	4.54	4.40	3.22
2	1	0	.043	—.030	.752	.0158	.014	4.30	4.13	4.15	2.94
3	1	0	.075	—.019	.751	.0214	.018	4.13	4.24	3.88	3.42
4	2	0	.121	—.009	.775	.0229	.023	3.70	3.86	3.38	2.57
5	4	0	.107	—.067	.802	.0287	.028	3.42	3.69	3.13	2.67
6	5	0	.112	—.086	.813	.0280	.030	3.18	3.20	2.88	1.88
7	9	0	.131	—.063	.836	.0266	.027	2.70	2.69	2.33	1.28
8	11	0	.122	—.058	.856	.0244	.022	2.54	2.12	2.38	1.10
9	14	0	.199	—.013	.867	.0236	.024	2.15	2.20	1.83	1.13
10	17	0	.274	+.009	.889	.0251	.026	1.82	1.67	1.63	.54
12	19	0	.242	—.089	.899	.0299	.030	1.70	1.67	1.67	.41
14	23	0	.321	—.015	.911	.0300	.027	1.46	1.59	1.23	.75
16	26	0	.394	+.010	.923	.0267	.027	1.17	1.44	1.08	.33
18	29	0	.408	—.020	.923	.0308	.030	1.17	1.15	1.09	.11
20	31	1	.538	+.046	.930	.0340	.032	1.03	1.09	.90	.18
25	33	1	.558	—.106	.945	.0324	.034	.74	1.13	.75	—.03
30	35	1	.648	...	.962	.0269	...	.53	.55	.52	—.22
35	38	1	.948	...	.974	.0250	...	.29	.14	.29	—1.31
37	39	1	1.000	all $\frac{0}{0}$	.975	.0250	...	.25	..	..	..
Selection up											
1	1	0	.000	—.075	.707	.0138	.015	4.93	4.79	5.27	6.07
2	1	0	.032	—.033	.681	.0197	.014	5.34	5.44	5.60	6.80
3	1	0	.015	—.100	.653	.0234	.026	5.73	5.57	5.90	6.53
4	1	0	.089	—.031	.648	.0289	.027	5.57	5.54	5.75	6.79
5	2	0	.125	—.028	.644	.0335	.035	5.51	5.63	5.98	6.81
6	2	0	.132	—.078	.616	.0485	.050	5.91	6.07	6.04	7.02
7	3	0	.178	—.048	.607	.0540	.054	5.91	5.96	6.31	7.61
8	3	0	.214	—.027	.596	.0574	.058	5.90	5.78	6.27	6.97
10	3	0	.211	—.069	.576	.0611	.068	6.20	6.07	6.60	7.06
12	3	2	.206	—.068	.543	.0654	.067	6.48	6.45	6.75	7.41
14	3	2	.193	—.124	.523	.0715	.079	6.87	7.00	7.04	7.78
16	3	2	.249	—.056	.519	.0736	.076	6.68	6.96	6.67	7.64
18	3	2	.252	—.059	.496	.0784	.078	6.89	6.87	6.96	8.05
20	3	2	.237	—.066	.485	.0715	.076	7.01	...	...	...
No selection											
1	1	0	.000	—.074	.731	.0156	.015	4.81			
2	1	0	.019	—.070	.722	.0170	.018	4.50			
3	1	0	.047	—.035	.738	.0186	.016	4.54			
4	2	0	.044	—.075	.727	.0232	.024	4.67			
5	3	0	.056	—.086	.724	.0279	.028	4.69			
6	4	0	.089	—.077	.726	.0296	.033	4.73			
8	4	0	.125	—.032	.723	.0347	.032	4.54			
9	4	0	.104	—.084	.719	.0362	.038	4.68			
10	4	0	.174	—.043	.707	.0398	.045	4.87			
∞	28	12	1.00	...	.7	.22	...	3.00			

* A few values for σ's of $Phen_g$ and $Phen_{g+e}$ in the unselected population: generation 0, .66 and 1.29; generation 10, .52 and 1.26; generation 18, .33 and .99; generation 20, .18 and 1.05; generation 37, zero and one.

The same generation 0 shown for the down selection also served for the other two experiments. All quantities calculated for all 40 loci except $\overline{F_i}$, which is the average for unfixed loci only.

DISCUSSION

Asymmetry in gene fixation

The observed asymmetry would be quite the expected result for major genes showing dominance without large nongenetic error. In such a case, selecting towards the dominant is constantly being retarded by the introduction into the selected population of recessive alleles carried by the heterozygote which is indistinguishable from the dominant homozygote, whereas in selection towards the recessive there is no difficulty in selecting recessives free of the dominant allele.

Dominance not the direct cause of the asymmetry: However, if there is large nongenetic variability so that the nongenetic error may be much larger than the phenotypic difference between recessive and dominant, the disadvantage of selecting towards the dominant is practically swamped out by the much greater disadvantage in selecting either way due to the poor ability to distinguish *any* genotype from any other. Then in selecting towards the dominant, one is continually introducing the recessive allele into the selected population not only by including the heterozygote but also recessive homozygotes which may happen to have suitable nongenetic errors. Similarly, in selecting towards the recessive, one is continually introducing the dominant allele into the selected population by including heterozygotes and dominant homozygotes. This selective disadvantage works equally for both directions of selection and can, if the nongenetic variability is sufficiently great, overwhelm the relative selective advantage of selecting towards the recessive. It is under such conditions of great environmental error in relation to phenotypic separation of genetic origin that symmetry in two-way selection is expected, whether there is directional dominance or not, provided the initial phenotype is midway between the extremes (FALCONER 1953). Our model has been chosen to meet these conditions by having a selection coefficient s of 0.27, which is small enough to meet FALCONER's condition, and an initial phenotype of 4.93 which is very close to the middle between zero and ten.

Explanation in terms of random drift as modified by selection: There seems to be a satisfactory qualitative explanation for the observed asymmetry in gene fixation, based on the contribution of the small population size to the situation. FALCONER's mathematical models were handled analytically and required the assumption of an infinitely large population. With small populations the allelic frequencies q_i are subject to appreciable fluctuations from sampling error. In each generation one is sampling the population allelic frequency by the use of just 12 individuals, each of which individually has an allelic frequency of either 0, 0.5 or 1. Therefore, the new population allelic frequency is unlikely to be very close to the old one. The resulting random fluctuations in each generation not infrequently build up into considerable movement of q_i in one or the other direction. If this drift, by chance, carries the q_i value to zero or one, that locus is permanently fixed, and this is the mechanism of gene fixation in breeding from small populations. Now obviously the closer q_i is initially to either zero or one, the greater the probability of fixation at that end. This was illustrated very well in the 120 gene model previously reported (L. M. ZUCKER 1960), where there was no selec-

tion and an initial $\overline{q_i}$ of 0.75; 21 loci were fixed as *gg* before the first locus was fixed as *GG*, corresponding to net drifts of 0.25 and 0.75 respectively.

In the present 40 gene model, therefore, even if there were no selection, the initial $\overline{q_i}$ of 0.7 would be expected to cause more rapid fixation at $q = 1$ (recessive end) than at $q = 0$ (dominant end). Two-way selection further widens this difference. In selection towards $q = 1$, the directed effect of selection is to move the q_i's ever nearer to one, making it increasingly more probable that the random undirected movement of the q_i's will cause fixation. In selection towards $q = 0$, the q_i's are first directedly moving towards the center of the range of q, where random drift has farthest to go before achieving fixation; it is only after q_i passes 0.5 that selection and drift reinforce each other. It will be noted that after 20 generations of selection towards $q = 0$ (towards the dominant, or up), $\overline{q_i}$ has just passed 0.5.

At first sight this effect is purely one of small population size as affected by selection and is quite unrelated to dominance; it seems purely fortuitous that in this experiment the more rapid gene fixation is associated with selection towards the recessive. Presumably, if we had started with $q = 0.3$ instead of 0.7, selection towards $q = 0$—i.e., towards the dominant—would be accompanied by early rapid fixation, whereas selection towards the recessive would show little gene fixation. However, it will be remembered that in the experiment q was placed asymmetrically at 0.7 because, granted directional dominance, this was necessary in order to get the phenotype into the middle of its range. With dominance, if phenotypic progress is to be symmetrical in the two directions, initial q is necessarily off center and early gene fixation is necessarily asymmetric. In the suggested case with $q = 0.3$, while it is true that gene fixation in the early phases of two-way selection would be principally associated with selection towards the dominant, this unnatural state of affairs would probably not continue for long. With q initially 0.3, the initial phenotype would be 0.91 (scale of zero to one). Following the rule that phenotypic progress falls off steadily as one approaches the phenotypic limit, further progress in selecting towards the dominant (phenotype of one) would be extremely slow compared with progress towards the recessive (phenotype of zero). Therefore, in selecting towards the recessive, q would very rapidly move, due to selection pressure, from the initial 0.3 through 0.5 and towards one. Rapid gene fixation would soon be expected to appear in association with selection towards the recessive as well as in continued association with selection towards the dominant.

Summarizing remarks: It appears that the relative rates of gene fixation in two-way selection in small populations are principally determined by the position of the initial q in relation to the limit values of zero and one, with selection towards the nearer limit accompanied by much greater fixation than selection towards the farther limit. A rate of gene fixation much faster than that to be found in the absence of selection may be associated with either selection towards the recessive or selection towards the dominant, according to which direction of selection represents the nearer end of the scale of allelic frequency. However, rapid gene

fixation associated with selection towards the dominant should be relatively rare, requiring as a condition that the initial phenotype be very close to the dominant phenotype.

Effects of selection on other consequences of moderate inbreeding—heterozygosis and allelic dispersion

We have just seen that selection can very considerably alter the rate of gene fixation induced by small population size. Similarly, it appears that selection can alter the course of the two other principal phenomena associated with small population size (moderate inbreeding); these are loss of heterozygosis and increasing dispersion of allelic frequencies due to drift.

Moderate inbreeding without selection: Both these phenomena can be quantitatively predicted in the absence of selection from the known population size and breeding plan. We showed previously, for a 120 gene model experiment without selection, that there was good agreement between the observed course of increase of σ_q^2 and the prediction from CROW's equation (CROW 1954)

$$(\sigma_q^2)_t = \frac{q(1-q)}{2N_e} + (1 - \frac{1}{2N_e})(\sigma_q^2)_{t-1}$$

Subscripts t and $t-1$ refer to the generation under consideration and the immediately preceding one; q is the initial allelic frequency, when $t = 0$ (unchanged, when there is no selection); N_e, the effective population size, is in our case 12 for the first generation of 12 drawn from an infinite population, and 2×12 thereafter for populations of 12 made up of equal offspring contributions from each breeding pair, rather than contributions following a Poisson distribution. Similarly, the expected change in the proportion of heterozygotes in the population could be calculated from WRIGHT's equations (see equations for H in LI 1955, p. 209, Table 59), with interpolation between the expectations for eight and 16 breeders. The ratio of this value to the Hardy Weinberg equilibrium value of $2q(1-q)$ measures the relative deficit of heterozygotes in the population. This quantity should be the same as $1-F$ (F is the inbreeding coefficient), and there was very good agreement between the calculated ratio of $H/2q(1-q)$ and observed values of $1-F$.

Moderate inbreeding with selection: Having explained all this for the case without selection, we can now proceed to a similar comparison for the present experiment with selection, as shown in Figure 3. On the left the points represent observed values of $1-F$ (from Table 1) for the two directions of selection; the heavy line represents the theoretical $H/2q(1-q)$ calculated as before, starting with the fourth generation. As before, there is initially a *rise* in heterozygosis due to mating the most distantly related (see L. M. ZUCKER 1960 for fuller discussion) before there is any common ancestry; the theory applies only after consanguineous mating begins to appear in the fourth generation. Heterozygosis appears to decline more rapidly than expected in the down selection experiment and less rapidly than expected in the up selection; indeed from the seventh to 20th genera-

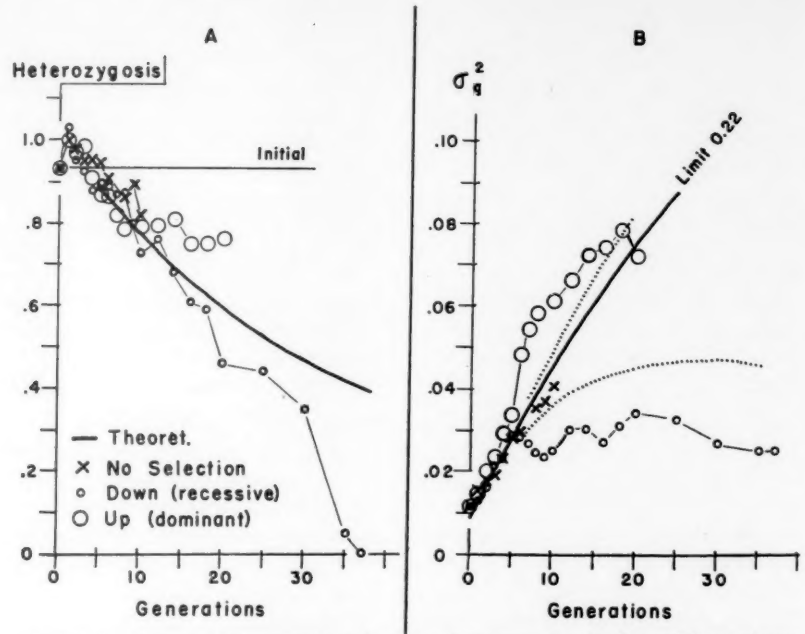

FIGURE 3.—Course of heterozygosis and course of variance of q in the model experiment compared with theory based on ancestry. The theoretical curve in A was calculated for $H_0 = 0.38$, $q_0 = 0.718$. The heavy curve in B was calculated for $q = q_0 = 0.718$, the two dotted curves for $q = q_t$.

tions of up selection there is no appreciable loss in heterozygosis. On the right of Figure 3, the points represent observed values of σ_q^2 for up and down selection. The theoretical course, calculated as in the previous report from CROW's equation, is shown by the heavy continuous line. The two dotted curves represent attempts to correct for changing q, due to selection, by using the observed q for each generation instead of the initial q. Even with this correction it still looks as though drift is far less than expected for selection down.

One cannot conclude that selection in one direction reinforces inbreeding effects, and in the other direction opposes them. Inbreeding is associated with gene fixation, with loss of heterozygosis, and with rise in variance of the allelic frequencies (random drift). In the up selection experiment the first two phenomena suggest *less* than expected inbreeding, but the high variance of q suggests *greater* than expected inbreeding. Similarly in the down selection, gene fixation and heterozygosis changes suggest *great* inbreeding; the low variance of q suggests *little* inbreeding. These divergent indications are of course in relation to theories which are not necessarily applicable when selection is imposed on inbreeding. The derivation of these theoretical expectations calls for mating the least related

individuals but with random choice among possible individuals of the same ancestry; we are choosing not at random but according to phenotype—that is, in part according to genotype. It is therefore possible to concentrate, or dilute, the proportion of genes identical by descent even though the formal mating scheme is maintained. It is of interest to compare the results with these theories partly because they are the only available reference standards, but more importantly because they are generally applied in practical breeding to small populations of domestic animals which are constantly under selection. The results suggest the possibility that inbreeding coefficients calculated from breeding plan or pedigree may, under these conditions of combined small population and selection, be quite far from the mark.

If we put aside the separate comparisons with (inapplicable) theory of the two phenomena of loss of heterozygosis and rise in allelic dispersion, and instead compare these two phenomena directly with each other, we find a direct and positive association. Table 1, columns 7 and 8, shows a close agreement between σ_q^2 and $(F-\overline{F_i})q(1-q)$. This relation appears to be an extension of the WRIGHT-WAHLUND equation $\sigma_q^2 = Fq(1-q)$ (see LI 1955, p. 298) for conditions under which our $\overline{F_i}$ would be zero; it has been discussed in more detail in the previous report (L. M. ZUCKER 1960). If a suitable theory is developed for the combined effects of selection and moderate inbreeding on either variance of q or on inbreeding coefficient, a theory for the other will follow from the above relationship.

Effects of moderate inbreeding on the progress of selection

We have so far been concerned with three phenomena which are basically due to breeding in small populations, and with how they are affected by selection. Now we come to the result of selection *per se*—the systematic change in phenotype—and will try to determine empirically how this is affected by the small population size, since a theoretical approach to the problem is not apparent. For this purpose we have derived a predicted course of phenotypic progress for the two-way selection which includes all the features of the model except the small population size.

Inbreeding depression: The prediction is represented by the two heavy curves in Figure 2A, and is actually a prediction for the quantity $(1-q^2)$ rather than what we have called Phen_{g+e}, the quantity comparable to what would be observed in an actual experiment. For a dominant gene with the population in Hardy Weinberg equilibrium, $(1-q^2)$ would be the same as the phenotypic value on a scale of zero to one; for our 40 gene model with each gene contributing 0.25 (on the scale of nongenetic variability), the value on the actual phenotypic scale would be $40 \times 0.25 \times (1-q^2)$ or $10 \times (1-q^2)$. But the experimental values of $(1-q^2)$, calculated from column 6 of Table 1 and plotted in Figure 2A as small circles, differ considerably from the observed phenotypic values taken from column 10 of Table 1 and plotted as large circles. This difference represents inbreeding depression. Because of inbreeding there is a relative deficit of heterozygotes (positive values of F); the shift of the alleles from two heterozygotes into

one recessive and one dominant homozygote causes a net drop from two large phenotypic contributions (2×0.25) to one large contribution (0.25), and hence depresses the average phenotypic value. The magnitude of this effect, $Fq(1-q)$, is greatest for a given F when $q = 0.5$, and hence greatest in the later stages of the up selection. Consideration of the remaining discrepancy between the theoretical curves and the observed course of $(1-q^2)$, which is presumably due to other features of the inbreeding, will be postponed until after a brief discussion of the derivation of the theoretical curves.

Expected rate of selection progress without inbreeding: One starts with the idea that the change in q, the allelic frequency, will proceed at the same rate for 40 identical genes as for a single gene of the same properties. This reduces the complexity of the problem considerably. FALCONER's (1953) analytical solution of the one gene problem is not applicable to our conditions even if there were no inbreeding because it involves mass selection; our model uses within-litter selection which can be expected to result in slower selection progress and requires an entirely different approach.

Let us consider the likelihood of making a favorable selection out of sets of four litter mates. Each individual is either genetically small (*gg*) or large (either *gG* or *GG*) with respect to the character under selection. The possible sets of four are shown in the first column of Table 2, designated as S or L. To calculate the change in q under selection it is necessary to know two sets of probabilities: (1) the probability of meeting each of the sets of four in the population presented for selection (shown in columns 2 and 3 for mass selection and within-litter selection), and (2) the probability of making a favorable selection from each kind of set (shown for down selection in the last column). The second set of probabilities is determined by the relation between the genetic and nongenetic contributions to the phenotype. An L will appear identical with an S if the L has an error of -0.25 while the S has an error of zero, or if the L has an error of -0.27, the S -0.02, etc. For each assumed error in L, there is a corresponding range of errors which the S may have and still be chosen in spite of the errors. We can assume ten equally probable errors for L, giving a standard deviation of about one, by

TABLE 2

Calculation of expected course of phenotypic progress

Possible sets of 4	Probability of occurrence of set: Mass sel.	Within-litter selection	Probability of favor. sel. down
4S, 0L	q^8	$q^4 + .0156q^2(1-q)^2 + .25q^3(1-q)$	1.00.
3S, 1L	$4q^6(1-q^2)$	$.1876q^2(1-q)^2 + q^3(1-q)$	.8113
2S, 2L	$6q^4(1-q^2)^2$	$.844q^2(1-q)^2 + 1.5q^3(1-q)$	.631
1S, 3L	$4q^2(1-q^2)^3$	$1.688q^2(1-q)^2 + q^3(1-q)$	.308
0S, 4L	Of no interest for selecting down		0

For within-litter selection, down: $SD = q^2(1-q)(.0955q + .2198)$

For mass selection, down: $SD = q^2(1-q^2)[.2452 + .2955(1-q^2)(1.054q^2 - .054)]$; $\Delta q = SD/(1 + q)$

These equations apply only for a phenotypic separation of 0.25, with the scale unit the nongenetic standard deviation, and for selection of 25 percent of the population.

dividing the area under a normal distribution curve into ten equal areas. The assumed errors in L are ±1.645, ±1.047, ±0.674, ±0.386, ±0.126. In a set of 3S, 1L, there will be satisfactory selection down as long as any of the three S's has an error "covering" any possible error in the L; if the L has an error of −1.645, favorable selection of the S will still be made if any one of the S's has a negative error > 1.395. The probability of this error for a single S is, from a table of normal areas, 0.5−0.4185 = 0.0815. The probability that the error of a single S does *not* "cover" this error in L is 0.9185. The probability that none of the three S's has a "covering" error is 0.9185^3 or 0.7749. The probability of the assumed error in L is 0.1, so that the combined probability of this assumed error in L, followed by selection of the L rather than any of the S's, is 0.07749. For each of the ten equally probable assumed errors in L, such a figure can be calculated, and their sum is 0.1887. This is the final probability of selecting a genetically large individual out of such a set when choosing the smallest phenotype. The probability of a favorable selection of one of the genetically small individuals is then 0.8113, and this is the figure shown in Table 2. The other cases are more lengthy but sufficiently straightforward arithmetic, and a similar set of probabilities can be calculated for selecting up.

Now it is possible to calculate the probability or frequency of S (genotype *gg*) in the selected population, as a sum of terms, one for each of the first four lines of Table 2, and obtained by multiplying column 3 by column 4 (for within-litter selection) or column 2 by 4 (for mass selection). This is an equation in q, a known quantity. Knowing $[gg]_{sel}$, and assuming that the ratio of $[gG]$ to $[GG]$ is the same in the selected as in the unselected population, it is possible to calculate q for the selected population. Since this must be the same as q for the offspring population, the derivation is complete. The resulting equations are shown in Table 2, and similar equations were derived for selecting up.

Mass selection was included in the derivation as well as within-litter selection because an independent derivation using an entirely different approach can be made for mass selection (based on FALCONER's analysis). It was very reassuring to find excellent agreement between the two predictions for mass selection, thus verifying the essential correctness of the figures of the last column in Table 2. Comparison of the predictions for mass and within-litter selection shows identical values of $(1-q^2)$ at generations 1, 2, 3 . . . 10 for mass selection and generations 2, 4, 6 . . . 20 for within-litter selection. This seems a likely consequence of the fact that all available genetic variance is utilized in mass selection, whereas only half the variance is used in within-litter selection.

Depressing effect of random drift on the progress of selection: Some of the discrepancy between the predicted and observed course of $(1-q^2)$ shown in Figure 2A may arise from a further effect of random drift. It is commonly assumed that successful solution of the one gene problem also solves the problem for any number of identical genes. This is technically true, but is actually not applicable in detail to small populations, where genes, initially identical, rapidly drift apart, as illustrated in Figure 1. Now it can easily be shown that phenotypic

progress for 40 genes each with a q_i of 0.7 will be much faster than phenotypic progress for 40 genes with widely dispersed q_i's *averaging* 0.7. From the equation of Table 2 one can calculate the change in q_i for one generation of selection for various values of q_i; these curves, which are very similar for up and down selection, are shown in Figure 2B. The greatest rate of phenotypic progress, as determined by the greatest Δq_i, is to be found at $q_i = 0.7$. (This corresponds to $(1-q_i^2) \cong 0.5$, and is the condition for the phenotype to be midway between the two extreme values.) Inspection of the curves makes it clear that with all 40 genes at 0.7, each will show maximal change in q_i with selection. If, on the other hand, the average $\overline{q_i}$ is still 0.7, but there is wide dispersion in individual q_i values, most of the loci will show less than maximal change, and the net change in $\overline{q_i}$ will be considerably less than maximal.

A distribution of q_i's can also be envisaged for which the average change under selection would be about the same whether the dispersion is wide or slight. This is the hypothetical case with all the q_i's distributed either from 0.7 to one, with average at about 0.85, or from 0.7 to zero, with average at about 0.4; then above average Δq_i contributions balance the underaverage ones. These two cases are the extreme possibilities. Random drift can never increase the rate of selection progress, but can cause a variable degree of depression ranging from negligible to considerable, depending on the form of the distribution of q_i. This effect can be an added source of asymmetry in two-way selection. It is also a reason why faster progress may be expected if one sets up a multiple cross for foundation stock than if one starts with a single stock which has been maintained in small populations (even if there has been no loss of genetic variability in such a stock due to actual gene fixation).

In summary, it appears that small population size can induce assymmetry in two-way selection both through the loss of heterozygosis and through random drift. The first effect leads to inbreeding depression, which is an effect limited to cases with directional dominance. The second (random drift) leads to variable depression in the rate of selection progress depending on the nature of the allelic distribution. This is an effect which will also appear in the absence of dominance. Since these combined effects of small population size have not been quantitatively evaluated, it is impossible to say whether there are still other effects contributing to the rate of progress, such as effects of dominance or further effects of inbreeding.

SUMMARY

A method has been explored for carrying on model breeding experiments combining moderate inbreeding (small population size) with selection for high and low values of a polygenic character subject to large nongenetic variation. The model studied has 40 initially identical genes, directional dominance (for high phenotypic values), and effective population size of 24. Two-way selection has been carried on for 20 or more generations, within litters, and with selection of 25 percent of each generation. The procedure yields complete information about genotypic distribution and nongenetic errors in both selected and unselected

populations, so that any population parameter of interest can be calculated. It could be readily adapted to nonidentical genes, multiple alleles, partial or overdominance, gene interaction, etc., and is probably well-suited to digital computer work.

Selection affects all three important consequences of small population size—gene fixation, loss of heterozygosis and allelic dispersion (random genetic drift). Selection may apparently reinforce one evidence of inbreeding and simultaneously oppose another. Inbreeding coefficients calculated from breeding plan or pedigree in small populations may be unreliable.

Small population size affects selection progress in several ways—through inbreeding depression, through the basically high rate of gene fixation, and through another consequence of random drift. This last effect operates to depress selection progress in both directions but to a variable degree, depending on the exact distribution of the allelic frequencies for the various loci concerned; it can be a source of asymmetry in two-way selection.

It appears that some kind of asymmetry in two-way selection is to be expected from dominant genes in small populations even under the most favorable conditions for avoiding it. In large populations one can presumably expect symmetry when the phenotype is midway between the extremes. But the necessary consequence of this—that the allelic frequency is asymmetrically located between its extreme values—leads in small populations to other asymmetric contributions to the selection.

Progress made per generation of mass selection requires two generations of within-litter selection.

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THE BEHAVIOR OF STRONG AND WEAK CENTROMERES AT SECOND ANAPHASE OF DROSOPHILA MELANOGASTER[1]

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Received October 5, 1959

THE contribution of the chromosome to its movements during the meiotic divisions has been the object of extensive genetic analysis. Past experiments (NOVITSKI 1952, 1955) have established that typical centromeres may be characterized as strong or weak by analyzing the behavior of anaphase bridges associated with these centromeres, and it has subsequently been shown that centromere strength (sometimes referred to as kinetic activity) is controlled by factors located in heterochromatic regions of the chromosome adjacent to the centromere (LINDSLEY and NOVITSKI 1958). In previous studies (NOVITSKI 1955; SANDLER 1954) it has been possible to measure centromere activity at the second anaphase of meiosis, but in neither of these investigations was it possible to test, at second anaphase, an array of centromeres (previously characterized by first anaphase tests as either weak or strong) under conditions duplicating the first anaphase configuration. This experiment describes a set of second anaphase bridges which simulate first anaphase configurations, and demonstrates that centromere activity is consistent at both meiotic divisions.

First anaphase bridges: The first satisfactory interpretation of the behavior of anaphase bridges in Drosophila was proposed by STURTEVANT and BEADLE in 1936. They suggested that at first anaphase single dicentric bridges formed by crossing over within X chromosome inversion heterozygotes stall on the spindle plate, and only noncrossover and double crossover chromatids reach the terminal pronucleus destined to become the functional egg nucleus. These considerations led to the theoretical expectation of two patroclinous males for every three double crossovers recovered in the male progeny from females heterozygous for a long X chromosome inversion; this ratio had been observed earlier (STONE and THOMAS 1935) and was confirmed by their own studies.

It was later established, however, that the behavior of anaphase bridges may be altered by changing their structure (NOVITSKI 1952). The chromosomes analyzed by STURTEVANT and BEADLE were ordinary X chromosomes with their typical subterminal centromeres, and the chromatid bridges resulting from exchange within the inversion were bounded near the extremities by the centromeres. If this configuration is altered by appending the intact long arm of the

[1] This work, performed at the Rocky Mountain Biological Laboratory, Crested Butte, Colorado, was carried out while the investigator held a National Science Foundation undergraduate research participantship awarded through the University of Oregon, and was supported in part by grant G-6202 from the National Science Foundation.

Y chromosome to both centromeres, the patroclinous males are virtually eliminated, that is, almost no nullo X eggs are produced, presumably because the bridges break or stretch such as to impart lethality to the egg pronucleus. The ratio of recovered double crossovers to patroclinous males in this case approaches 3:0. Should only one centromere bounding the bridge carry this extra arm, the observed ratio is 3:1, and it is concluded that the centromere with the attachment (i.e., the strong centromere) drags the dicentric to one of the terminal nuclei, thus halving the number of patroclinous males expected from simple inversion heterozygotes. Centromeres on opposite ends of a double chromatid bridge which stalls, producing nullo X eggs, are termed weak; those centromeres which stretch or break the bridge and eradicate the patroclinous male class are termed strong.

Finally, further analysis has demonstrated that the strength of any centromere, regardless of its origin, is determined by genetic factors located in the physically adjacent heterochromatin. A simple rule is that centromeres attached to the X chromosome and carrying a complete long arm of the Y chromosome, either as an extra arm or as an interstitial addition to the X chromosome, are usually strong; those carrying no extra arm are usually weak (LINDSLEY and NOVITSKI 1958). Figure 1 schematically demonstrates the behavior of first anaphase bridges bounded by strong and weak centromeres.

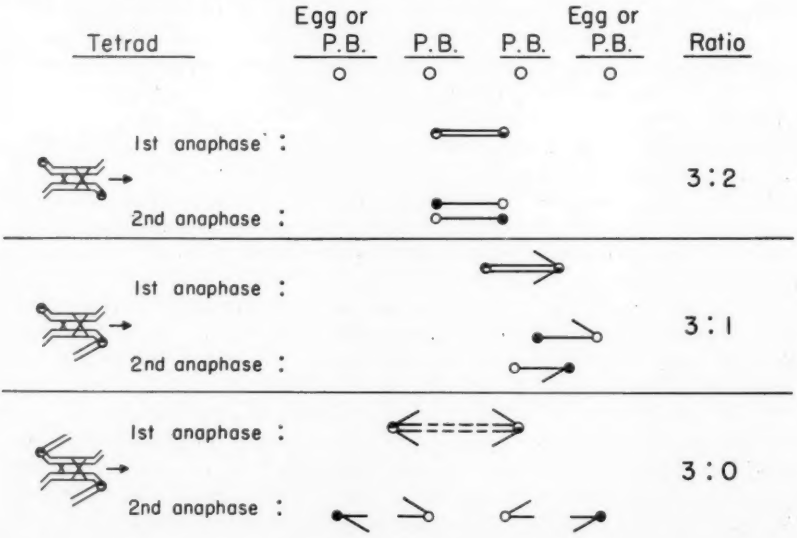

FIGURE 1.—The kinds of bridge configurations resulting from four-strand double crossing over in flies heterozygous for large X chromosome inversions. The centromeres with no extra arm behave in a typically weak fashion; those with the extra arm behave as strong centromeres. The ratio of recovered double crossovers to patroclinous males is represented for each case.

Second anaphase bridges: The analysis of the activity of second anaphase bridges has been less direct and complete than that of first anaphase bridges. STURTEVANT and BEADLE (1936) showed that second anaphase bridges arising from exchanges in the tandem metacentric compound (tandem attached-X) produce lethal eggs. The crossovers under consideration were singles and three-strand doubles, each of which forms second anaphase ties having a single strand between two sister centromeres and also an entire X chromosome appended to each centromere. It has since been demonstrated, however, that while these bridges do not hold at second anaphase, others of different chromatid structure stall and produce nullo X eggs (NOVITSKI 1955). First, one of the two different types of four-strand double exchanges in the tandem metacentric produce second anaphase bridges consisting of two chromatids of equal length tying sister centromeres together. This configuration, apparently not considered by STURTEVANT and BEADLE, holds at second anaphase, and nullo X eggs are produced. On the other hand, an analysis of the products of exchange in the tandem ring show that, at second anaphase, when the two chromatids connecting sister centromeres are of unequal length, almost no nullo X eggs are produced. SANDLER (1957) has shown the same to be true for reversed compound rings. It is argued that when the centromeres are connected by two strands of unequal length, they are sufficiently strong to "break" one strand at a time; but when two strands of equal length bind the centromeres, the bridges hold. It has not been possible, however, to construct second anaphase bridges with structures similar to those of first anaphase bridges.

Consequences of crossing over in dl-49 heterozygotes: The experiment described here arose from a consideration of data obtained by FORBES (1958) who has kindly consented to a discussion of his findings. It was observed that flies heterozygous for the short, medially located X chromosome inversion dl-49, as well as heterozygous for large inversions in the major autosomes, produced a high frequency of patroclinous males (0.91 percent), but a much lower frequency of matroclinous females (0.02 percent), indicating that primary nondisjunction probably was not responsible for the high frequency of exceptional males. It was suggested by PROFESSOR EDWARD NOVITSKI that such males might arise from a double exchange, one within the inversion and the other without, and proximal to the inversion. This three-strand double exchange (STURTEVANT and BEADLE 1936) allows homologous centromeres to separate at first anaphase, but binds sister centromeres by an X chromatid at second anaphase. This result was observed cytologically in Trillium by SMITH (1935). Similar dicentrics, at first division, are known to be eliminated from the egg pronucleus as described previously; it was proposed that nullo X eggs might be produced by a similar elimination of these bridges at the second anaphase division. Thus, in order to test the activity at second anaphase of centromeres of known first anaphase strength, this experiment utilized the dl-49 configuration to produce (1) a set of second anaphase bridges with subterminal, or weak, centromeres, and (2) a set of second anaphase bridges bounded by strong centromeres, that is, centromeres carrying the long arm of the Y chromosome (Y^L). It was predicted that the former would produce a high frequency

of patroclinous males, while the latter would yield a greatly reduced number of patroclinous males.

EXPERIMENTAL PROCEDURES

The desired crossover is a three-strand double, one exchange within the inversion and one without and proximal to the inversion. One set of experiments was established with this X chromosome configuration: dl-49·/X·; another with this configuration: dl-49·Y^L/X·Y^L, where the dot indicates the location of the centromere. Figure 2 illustrates the three-strand double exchange and the resulting second anaphase bridges. All females were heterozygous for the autosomal inversions associated with Curly on the second chromosome and with Dichaete on the third in order to increase the frequency of exchanges in the X chromosomes. Two different chromosomes with the long arm of the Y attached were used; these had been previously tested in first anaphase bridges and proved to be strong. These results are listed in Table 1 of Novitski (1952) as detachments A-2 and U-8e. The mutants marking these chromosomes and thereby facilitating the construction of females heterozygous for dl-49, with and without Y^L, are not detailed here because they are irrelevant to the results presented below. The male progeny of the group of crosses involving X chromosomes carrying Y^L were tested with the Y″ (iso Y^s) chromosome for fertility, thus verifying the presence of Y^L. In one experiment, crossveinless and vermilion were heterozygous so that it was possible to check on the frequency of double exchanges occurring within the dl-49 inversion. To insure that any difference between the two kinds of experiments would be

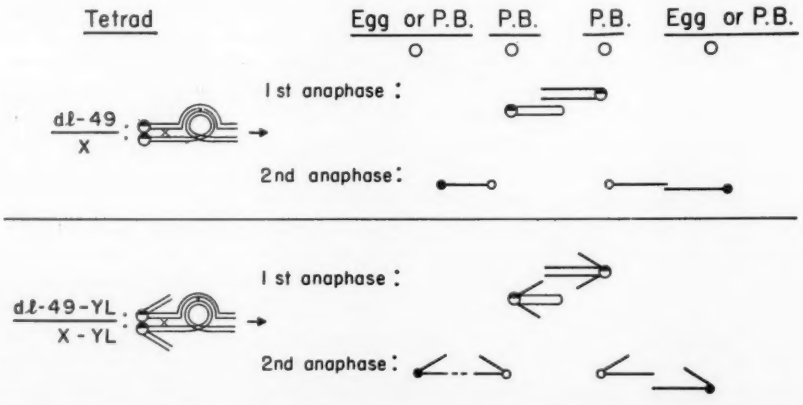

FIGURE 2.—The two types of second anaphase bridge configurations resulting from three-strand double exchanges in dl-49 inversion heterozygotes. One exchange is within the inversion, the other proximal to the inversion. The first bridge configuration holds and produces nullo X eggs. The second configuration, characterized by the appended arm, delivers lethal fragments to the eggs.

TABLE 1

Progeny from crosses of dl-49 inversion heterozygotes, with and without Y^L appended to the X centromeres

Genotype		Progeny: Exceptions: Females: No.	Females: Percent	Males: No.	Males: Percent	Total no. of flies observed
dl-49·/X·;	Exp. 1	1	0.02	48	0.91	5268
Cy/+;*D*/+	Exp. 2	0	0.0	11	0.89	1246
	Exp. 3	0	0.0	7	1.00	697
	Total	1	0.01	66	0.92	7211
dl-49·Y^L/X·Y^L;	Exp. 4	0	0.0	5	0.12	4323
Cy/+; *D*/+	Exp. 5	0	0.0	7	0.24	2896
	Total	0	0.0	12	0.17	7219

The dot indicates the location of the centromere. The X chromosomes tested in Experiments 1, 2 and 3 are of independent origins, as are the X·Y^L detachments tested in Experiments 4 and 5.

a function of the different centromere regions used rather than of some peculiarity of any one chromosome or centromere region, three different X centromeres were used in the three experiments without Y^L, and two different Y^L detachments were used in the two experiments with Y^L.

RESULTS

Table 1 presents the data from five experiments involving X chromosomes of different origins. Those females of configuration dl-49·/X· produced a high frequency of patroclinous males (avg. 0.92 percent) but a low frequency of matroclinous females (avg. 0.01 percent). Females of the second configuration, dl-49·Y^L/X·Y^L, produced a greatly reduced number of patroclinous males (avg. 0.17 percent) and no matroclinous females. Further, the male progeny of Experiment 3 was examined for the presence of double crossover chromosomes; out of a total of 697 flies, no double crossovers were recovered.

DISCUSSION

The patroclinous males observed here were produced from nullo X eggs which might possibly have arisen in the following four ways: (1) primary nondisjunction of the maternal X chromosomes, (2) loss of an X chromosome at either first or second division, (3) double chromatid bridges at first anaphase arising from four-strand double exchanges within the dl-49 inversion, and (4) second anaphase bridges arising from three-strand double exchanges as diagrammed in Figure 2. The contribution to the patroclinous male class from mechanism 1 may be assessed directly by the frequency of matroclinous females recovered from double-X eggs. The frequency of such exceptional females is extremely low, and it is clear that primary nondisjunction produced few patroclinous males. Mechanism 2 is un-

likely because it does not account for the discrepancy in frequency of patroclinous males between the configurations carrying Y^L and those without Y^L, nor does it explain the great reduction in frequency of the exceptional males when no inversions are carried in the autosomes (FORBES 1958). The number of patroclinous males arising from first anaphase bridges, possibility 3, is determined by the frequency of four-strand double exchanges within the dl-49 inversion. Such exchanges produce two patroclinous males to every three double crossover chromatids recovered (STURTEVANT and BEADLE 1936). Since the number of double crossovers recovered in Experiment 3 was zero, it may be concluded that doubles within dl-49 occurred very rarely if at all, and no significant number of nullo X eggs was produced from first anaphase bridges. This result is in agreement with the observations of others that double exchanges do not occur in dl-49 inversion heterozygotes even in the presence of the *Cy* and *CxD* inversions (NOVITSKI 1950).

It is clear that the large number of patroclinous males observed in the progeny of females heterozygous for dl-49, *Cy*, and *CxD* inversions result from the frequent failure of passage to the functional egg nucleus of an X chromatid or X chromatid fragment at second anaphase. The virtual disappearance of this class (when Y^L arms are added to the bridge centromeres) demonstrates that the activity of typical first anaphase strong and weak centromeres extends, with full integrity, to second anaphase.

It should be pointed out that while the terms "fragment" and "break" have been used previously in describing the behavior of anaphase bridges bounded by strong centromeres, there is no cytological evidence for actual fracture of the chromosomes in Drosophila. HINTON and LUCCHESI (1960) have identified cytologically first anaphase bridges formed by crossing over within ordinary X chromosome inversion heterozygotes and also second anaphase bridges formed by crossing over within the tandem metacentric. However, it was impossible for these investigators to achieve sufficient resolution to distinguish chromosome breakage from chromosome attenuation. The breaks discussed in this and previous studies are defined only genetically by the deviations observed in the frequency of the patroclinous male class from that expected from simple X chromosome inversion heterozygotes. This genetic observation might be the consequence of a great attenuation of the bridges at anaphase which could impart lethality by, for example, disrupting the formation of an intact nuclear membrane. The Drosophila bridges most certainly do not simply snap without uncoiling or stretching, for this would probably lead to a preferential inclusion of lethal fragments in the egg nucleus. NOVITSKI (1951) has shown that when two structurally different chromatids compete for inclusion in the functional nucleus, the shorter fragment is recovered with greater frequency than the longer. Since, however, there is no loss of the double crossover and presumably the noncrossover chromatids which are carried at the extremities of a first anaphase bridge arising from single or three-strand double exchanges, the conclusion is that the chromatid bridge breaks,

if at all, only after stretching sufficiently to orient the intact chromatid to the nucleus.

This suggestion agrees well with what has been observed cytologically in *Sciara impatiens* inversion heterozygotes. CARSON (1946) observed that dicentric bridges in Sciara become considerably attenuated at the meiotic divisions, and that breakage, which occurs occasionally, does not interfere with the directed segregation of the intact chromatids to the terminal nuclei. A consistent picture of bridge behavior in Drosophila may then be provided if we assume that the process of attentuation and occasional breakage imparts lethality to the terminal nuclei only in those cases where there is no intact X chromatid to be oriented into the nucleus. Such is the case with, for example, second anaphase bridges bounded by centromeres carrying Y^L. On the other hand, where first anaphase bridge centromeres carry an intact X chromatid which may separate at the second division from the dicentric, these chromatids are directed to the terminal nuclei. Finally, when the bridges are bounded by subterminal (weak) centromeres, the dicentrics presumably are not greatly stretched and simply do not reach the functional nucleus. Such is the case with, for example, second anaphase bridges bounded by weak centromeres and also with double first anaphase bridges formed from simple X chromosome inversion heterozygotes.

SUMMARY

A genetic analysis of the behavior at second anaphase of centromeres of known first anaphase strength is presented. It is shown that centromere activity, controlled by factors located in the heterochromatin of the anaphase bridge with which the centromere is associated, is consistent at both meiotic divisions. A scheme is presented to provide a consistent explanation for the behavior of the various kinds of bridges known at first and second anaphase.

ACKNOWLEDGMENT

The author would like to extend thanks to PROF. EDWARD NOVITSKI for his guidance throughout the course of this work, and to DRS. WILLIAM K. BAKER and DANIEL L. LINDSLEY for their helpful comments concerning the manuscript.

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CONTENTS OF PRECEDING ISSUES

JANUARY, 1960

CASPARI, ERNST W., Richard B. Goldschmidt.

New Policies for Genetics.

MIYAKE, T., Mutator factor in *Salmonella typhimurium*.

GREEN, M. M., Double crossing over or gene conversion at the white loci in *Drosophila melanogaster*?

ASHMAN, R. B., Stippled aleurone in maize.

MILKMAN, ROGER D., The genetic basis of natural variation. I. Crossveins in *Drosophila melanogaster*.

HASHIMOTO, KAZUO, Streptomycin resistance in *Escherichia coli* analyzed by transduction.

RHYNE, CLAUDE L., Leaf lobation in Gossypium amphidiploids as determined by leaf-shape genes of Gossypium diploid species.

LEWIS, C. F., and T. R. RICHMOND, The genetics of flowering response in cotton. II. Inheritance of flowering response in a *Gossypium barbadense* cross.

HINTON, CLAUDE W., and JOHN C. LUCCHESI, A cytogenetic study of crossing over in inversion heterozygotes of *Drosophila melanogaster*.

STALKER, HARRISON D., Chromosomal polymorphism in *Drosophila paramelanica* Patterson.

PETERSON, PETER A., The pale green mutable system in maize.

FEBRUARY, 1960

PARKER, D. R., The induction of recessive lethals in Drosophila oocytes.

GOWEN, JOHN W., A Drosophila intersex-triploid.

KREIZINGER, JEAN D., Diepoxybutane as a chemical mutagen in *Zea mays*.

HAYMAN, B. I., The theory and analysis of diallel crosses. III.

SCHEINBERG, S. L., Genetic studies of serum antigens in species hybrids.

OGUR, M., S. OGUR, and R. ST. JOHN, Temperature dependence of the spontaneous mutation rate to respiration deficiency in Saccharomyces.

MAGUIRE, MARJORIE P., A study of homology between a terminal portion of Zea chromosome 2 and a segment derived from Tripsacum.

HUBBY, J. L., and H. S. FORREST, Studies on the mutant maroon-like in *Drosophila melanogaster*.

KIDWELL, J. F., H. J. WEETH, W. R. HARVEY, L. H. HAVERLAND, C. E. SHELBY, and R. T. CLARK, Heterosis in crosses of inbred lines of rats.

STIMPFLING, J. H., and M. R. IRWIN, Gene homologies in Columbidae.

MARCH, 1960

SPRAGKLING, LE ETTA SANGER, The chromosome complement of the developing eggs produced by *Drosophila parthenogenetica* Stalker virgin females.

BROSSEAU, GEORGE E., JR., Genetic analysis of the male fertility factors on the Y chromosome of *Drosophila melanogaster*.

SLATIS, HERMAN M., An analysis of inbreeding in the European bison.

SAND, SEAWARD A., ARNOLD H. SPARROW, and HAROLD H. SMITH, Chronic gamma irradiation effects on the mutable *V* and stable *R* loci in a clone of Nicotiana.

TSUKAMURA, MICHIO, Relationship between the intracellular localization of beta-radioisotopes and their mutagenic effect.

SCHNICK, SHIRLEY MCALPINE, TERUMI MUKAI, and ALLAN B. BURDICK, Heterozygote viability of a second chromosome recessive lethal in *Drosophila melanogaster*.

BEATTY, ALVIN V., and JEANNE W. BEATTY, Postirradiative effects on chromosomal aberrations in Tradescantia microspores.

WEAVER, ELLEN C., Somatic crossing over and its genetic control in Drosophila.

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